ARCHIVES ITALIENNES DE BIOLOGIE

FONDÉES PAR ANGELO MOSSO
ET CONTINUÉES PAR VITTORIO ADUCCO

PUBLIÉES PAR GIUSEPPE MORUZZI
Professeur de Physiologie à l'Université de Pise

TOME XCIX – Fasc. 4
Paru le 10 Novembre 1961

PISA
UNIVERSITÀ DEGLI STUDI
1961

RECOMMANDATIONS AUX AUTEURS

Les *Archives italiennes de Biologie* publient des travaux originaux de physiologie, en français ou en anglais, selon le choix de l'auteur. Des travaux de tous pays peuvent être acceptés, à condition qu'ils n'aient pas été publiés dans un autre journal. Toutefois des travaux ayant paru seulement en italien, pourront être traduits et publiés dans les *Archives italiennes de Biologie*, à la requête de la Rédaction. Dans ce cas l'indication bibliographique du travail italien sera donnée.

Les manuscrits dactilographiés doivent être envoyés à la Rédaction des *Archives italienne de Biologie* – Via S. Zeno, 13 – Pisa (Italie). Pour les citations et le résumé les Auteurs sont priés de prendre comme modèle les travaux publiés dans ce fascicule. Les photographies de chaque figure seront faites sur papier glacé. Dès qu'un travail est accepté, l'auteur est informé du prix des figures et des tirés à part.

Les livres pour les analyses doivent être envoyés à: Dr. M. G. F. Fuortes, National Institute of Health, Bethesda 14, Md., U.S.A.

Chaque volume comprendra quatre fascicules. Le prix d'abonnement est de 10 $ U. S. A. et devra être envoyé à l'Administration des « Archives italiennes de Biologie» (F. Vallerini, « Sala delle Stagioni » - Lungarno Pacinotti, 1 - Pisa, Italie). (Virement postal N. 22/4955).

The *Archives italiennes de Biologie* are devoted to publication of original contributions to physiology, in either French or English, according to the choice of the Author. Papers from any Country will be considered for publication on the understanding that they have not been published in another journal. However papers which have been published only in Italian may be translated and published in the *Archives italiennes de Biologie* on the request of the Editorial Board. The Italian reference will then be given in a foot note.

Type written manuscripts should be sent to the Editor of the *Archives italiennes de Biologie* – Via S. Zeno, 13 – Pisa (Italy). For citations to literature, references and summary the Authors are asked to adhere strictly to the pattern utilized in the papers published in this issue. Illustrative material should be in the form of photographic reproduction on glossy paper, suitably arranged and mounted to form a complete figure. When a manuscript is accepted, the author will be notified of the cost of the plates for illustrations and of the reprints.

The books to be reviewed should be sent to: Dr. M. G. F. Fuortes, National Institute of Health, Bethesda 14, Md., U.S.A.

Each annual volume will appear in quarterly issues. Annual subscription rate, payable in advance, is U. S. A. $ 10. Remittances should be sent to: The Administration, « Archives italiennes de Biologie » (F. Vallerini, « Sala delle Stagioni » - Lungarno Pacinotti, 1 - Pisa, Italy).

This issue of the ARCHIVES ITALIENNES DE BIOLOGIE completes vol. 99 for the year 1961. The subscription rate for vol. 100 (1962) has not changed and remains at $ 10.00 U.S.A. or the equivalent in any currency. All remittances should be sent to: The Administration, « Archives italiennes de Biologie », F. Vallerini, Sala delle Stagioni, Lungarno Pacinotti, 1 – Pisa, Italy.

THE LOCAL CORTICAL RESPONSE IN THE HIPPOCAMPUS OF RABBIT

P. ANDERSEN AND J. JANSEN JR.
Neurophysiological Laboratory, Anatomical Institute, University of Oslo, Norway

INTRODUCTION

Since the pioneering study of Adrian (1) the neocortical response to an electrical stimulus applied near the recording electrode has been the subject of a long series of investigations (for reviews see 9, 26). However, the interpretation of this local cortical response is still a matter of considerable controversy (9). The complex histological arrangement of the neocortical neurons and their afferent and efferent axons is perhaps the main obstacle for an unequivocal analysis of this response. On the other hand, the architecture of the hippocampal cortex is much more uniform, and has been described histologically in great detail (13, 24).

The purpose of the present study has been, first to take advantage of this relatively simple cortex in an investigation of the site of initiation and the propagation of impulses along the neuronal surface in response to a direct electrical stimulus; second to correlate the local hippocampal response with the corresponding neocortical potential.

The results confirm and extend the results obtained by Cragg and Hamlyn (16) who studied the effect of intracortical stimulatio on the neurons of the hippocampal field CA1 (for anatomical data see 3).

METHODS

Seventeen adult rabbits were used. The animals were anesthetized with urethane-chloralose (750 mg/kg and 40 mg/kg, respectively), 2/3 of the dose given intravenously and 1/3 intraperitoneally.

In the experiments with anoxia, the animals were immobilized by intravenous decamethonium bromide (Decacurin AFI) 0.25 mg/kg. Artificial respiration was carried out through a tracheal cannula with a vacuum-driven respirator. Pure oxygen was supplied under positive pressure (10 cm H_2O). Anoxia was produced by substituting oxygen with nitrogen in the respirator.

The dorsal aspect of the hippocampus was exposed by suction of the overlying neocortex. Only the hippocampal field CA1 of Lorente de Nó (24)

was studied in detail. The exposed cortex was irrigated with warm Ringer's solution. The temperature of the recording room was about 25° C.

Stimulation was carried out with bipolar electrodes, usually applied to the cortical surface. In some experiments a stainless steel electrode with an interpolar distance of 1 mm was employed. In most experiments the electrode consisted of two copper wires of 0.2 mm which were kept close together with an insulating varnish. For depth stimulation two insulated, stainless steel wires of 35 μ thickness were similarly lacquered together. The stimulators employed produced square wave pulses with independently controllable frequency, pulse duration and voltage. The usual shock repetition rate was 0.3/sec and pulse duration 0.1 msec.

Records from the surface were obtained by a silver ball monopolar electrode (0.3 mm). The large indifferent electrode was placed on the cut edge of the skin in the neck. For depth recording glass capillaries of 5-10 μ, filled with 3 M KCl or 3 M NaCl were employed. They were carried by a micromanipulator allowing graded vertical movements of 1 μ, and inserted approximately perpendicularly to the cortical surface. The estimation of the actual extent of electrode penetration was a difficult problem. The change in response pattern and the micromanipulator readings were compared on inserting and retracting the electrode. The sequence of events was identical in the two cases. In relation to the micromanipulator readings, however, the changes in response occurred deeper in the cortex during the insertion than during retraction of the electrode. This difference was usually about 100 μ and indicated that the cortex was somewhat compressed, respectively lifted by the electrode movements.

The recording electrodes were connected through an external cathode follower to a four stage push-pull amplifier. The impulses were visualized on a double beam cathode-ray oscilloscope with sweeps synchronized with the stimulus. The time constant of the recording system could be varied between 10 and 200 msec. The form of the response was controlled with a DC amplifier.

Histological sections (2) were prepared approximately parallel to the electrode track to study their extent and the relation of the microelectrode recordings to the layers of the CA1 cortex.

Results

Form and threshold of the local hippocampal response. — The typical response of the field CA1 of the rabbit hippocampus to direct electrical stimulation of the cortical surface near the recording electrode consisted of an initial surface negative spike followed by a negative wave, sometimes with superimposed small spikes (Fig. 1 *A* and *B*). This potential complex will be called the local hippocampal response.

The initial spike was usually purely surface negative, and only occasionally preceded by a small positivity. The duration of the negative phase measured 1-4 msec, usually about 2 msec. The following surface negative wave lasted considerably longer, from 25-60 msec. A small negative crest was often observed in the start of the rising phase of this component. Following stronger stimulation, or in periods of increased cortical excitability, one or more negative spikes could be elicited superimposed upon the negative wave (Fig. 1 *B*). These spikes were usually of rather small magnitude.

If the recording electrode was moved a short distance outside the most responsive area, the longlasting negative wave was changed to a triphasic negative-positive-negative wave (Fig. 1 *D*), or even a purely positive wave (Fig. 1 *C*). Such a change of the polarity of

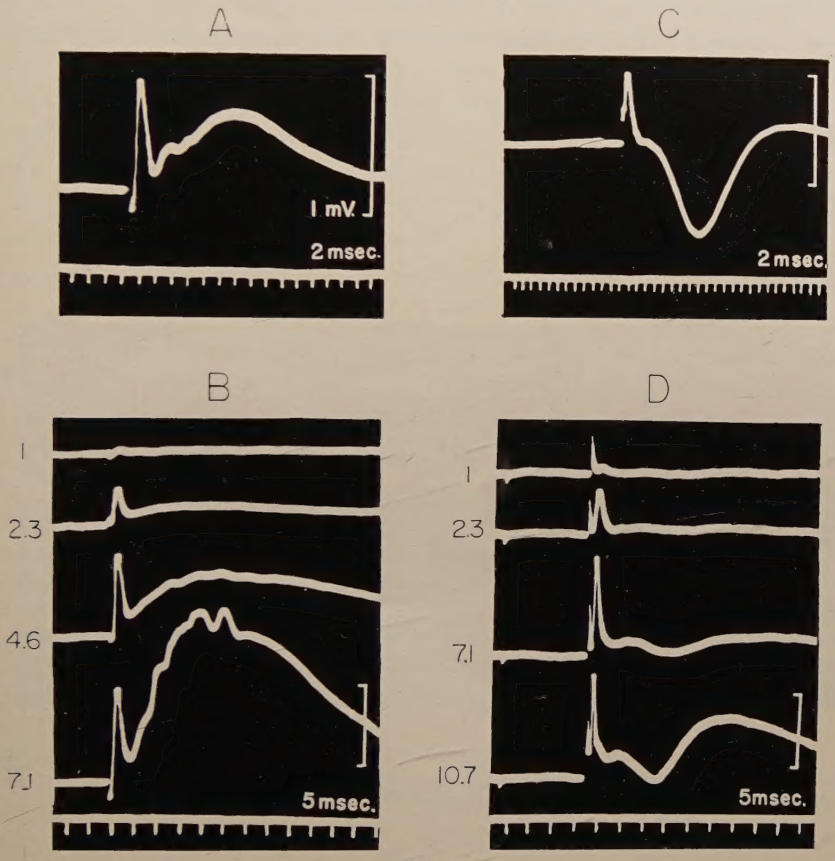


Fig. 1. – *Local hippocampal responses.*

A: most usual form of the response. *B*: effect of increasing stimulus strengths indicated in multiply of threshold to the left of each record. *C*: response with a purely positive slow wave. *D*: response with negative-positive slow wave and effect of increasing stimulus strengths as in *B*.

the negative wave was also observed when the hippocampal cortex showed signs of depressed excitability.

The threshold of the initial spike was similar to or slightly lower than that of the following negative wave (Fig. 1 *B* and *D*). The threshold of the late small spikes was definitely higher.

Distribution of the local hippocampal response. — The distribution of the responses following stimulation of a given point on the hippocampal surface is illustrated in Fig. 2. The activated region comprised a wedge-formed area extending rostrally and slightly laterally from the stimulated point towards the fimbria. Only small deflections were observed in the opposite direction. The amplitude of the initial

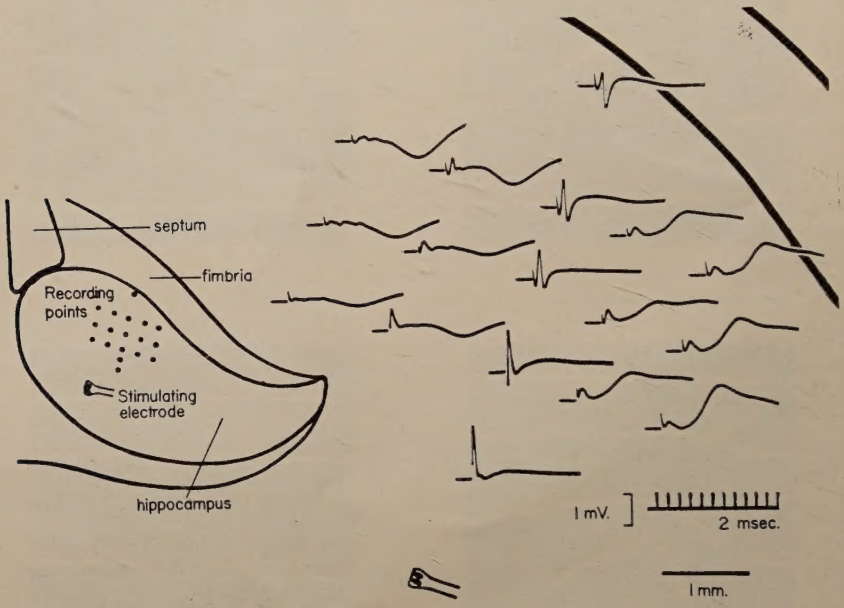


Fig. 2. – *Distribution of local response on the hippocampal surface.*

Schematic drawing to the left indicate localization of stimulating and recording electrodes. Corresponding responses shown to the right. No responses were obtained in the opposite direction.

spike as well as that of the following wave diminished with increasing distance from the stimulating electrode.

The distribution of the negative wave was not identical to that of the initial spike. Thus, the negative wave was obtained from more extensive zone than the initial spike, the former exhibiting its maximal amplitude slightly lateral to the area of maximal spike responses. When recording a little outside the area of maximal responses, the amplitude of the initial spike decreased rapidly, and the polarity of the following negative wave was often partially (Fig. 1 *D*) or totally (Fig. 1 *C*) reversed.

With increasing distance from the stimulated point, the latency of the initial spike increased. The average conduction velocity was estimated in five experiments to about 2.5 m/sec (1.3-3.3 m/sec).

Section of the alveus. — In order to localize the fibres mediating the impulses responsible for the local hippocampal response a shallow cut, severing the alveus fibres, was made between two recording electrodes. This section reduced the response obtained from the distal electrode-b (lower lines in Fig. 3). On the proximal side of the lesion a qualitatively unchanged response was still obtained (B) showing that the result was not due to a general loss of responsiveness of the cortex. The results suggests that the impulses are traversing in axons situated within the alveus.

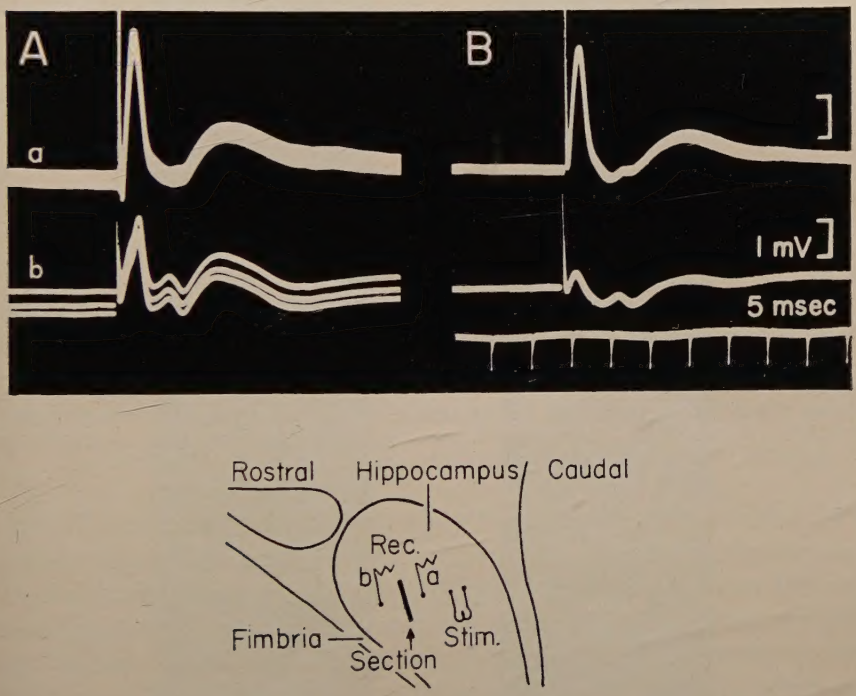


Fig. 3. – *Effect of section of alvear fibres.*

Electrode arrangement as indicated in diagram below the recordings. Upper trace in each record obtained from the proximal recording electrode *a*, while lower trace is obtained from distal electrode *b*. *A*: control before lesion. *B*: records obtained following shallow (less than 0.5 mm deep) section between the two recording electrodes, severing the superficial alvear fibres. Three superimposed sweeps in each record.

Stimulation at different depths. — The threshold and the amplitude of the initial spike of the local hippocampal response changed in a reproducible manner when the stimulating electrode was inserted through the various cortical layers. The lowest threshold and the largest evoked responses were observed when the tip of the stimulating electrode was 0.5 mm deep to the ependymal surface of the CA1 (Fig. 4). This depth roughly corresponds to the pyramidal cell layer.

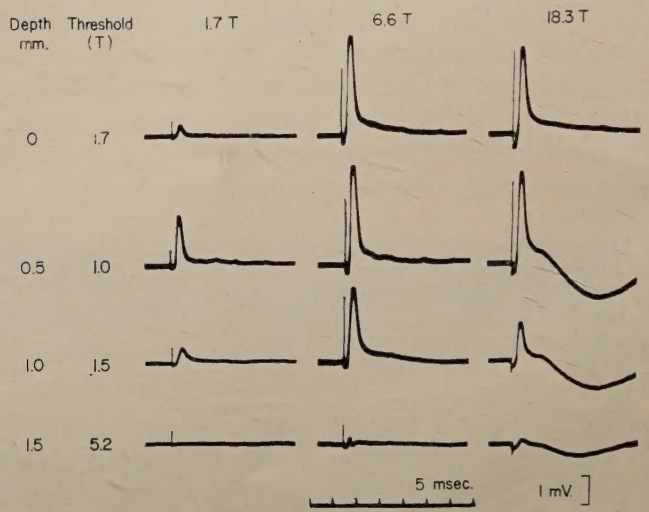


Fig. 4. – *Surface responses to stimulation at different depths and at different stimulus strengths.*

Only initial spike is recorded. Left column of numbers indicates depth of stimulating electrode for the corresponding responses. Second column shows the relative threshold of the initial spike evoked from corresponding depths. Numbers at the top indicates the stimulus strengths in multiply of threshold (T) for the corresponding vertical column of responses.

Fig. 4 shows the importance of using low stimulus intensities in such measurements. By use of stronger stimuli (6.6 and 18.3 × threshold) the differences between the amplitudes of the responses elicited from the various cortical layers are less clear. These results suggest that the electrical stimulus primarily excites the cell bodies and/or the proximal part of the apical dendrites of the pyramidal neurons. Stronger shocks in addition probably stimulate the axons coursing in the alveus near the ventricular surface.

Recording at different depths. — Fig. 5 illustrates two typical experiments in which records were obtained by a glass capillary electrode penetrating the CA1 cortex parallel to the pyramidal apical

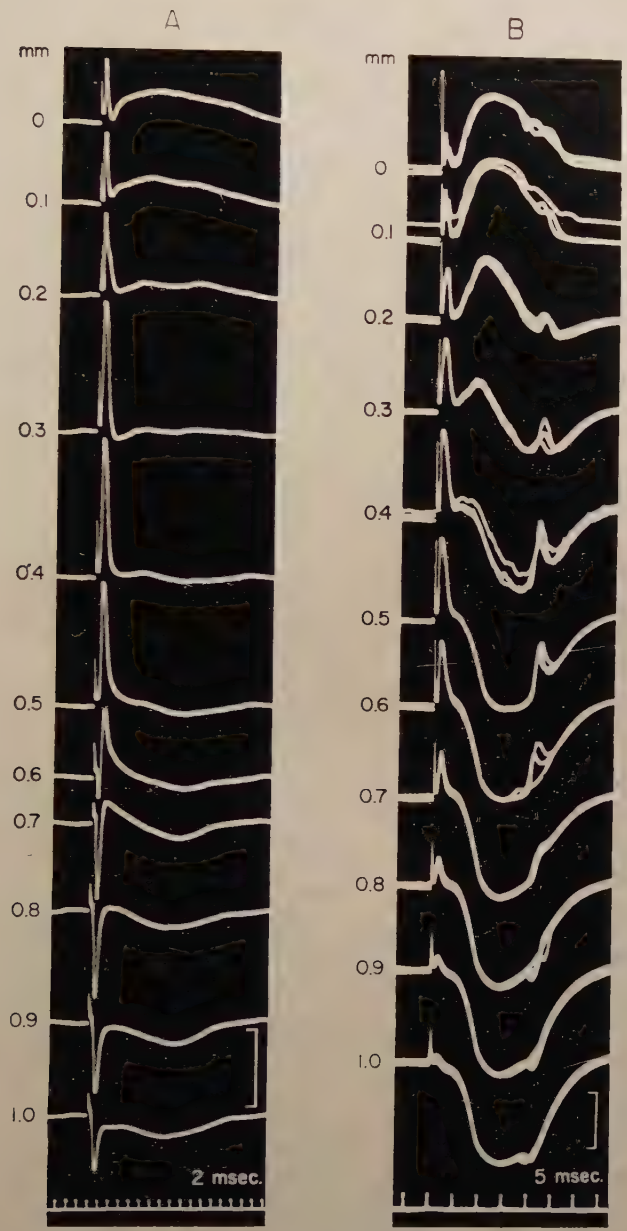


Fig. 5. – *Responses recorded at different depths of the hippocampal cortex.*

A and *B* from two different experiments. Further explanation in text.

dendritic shafts. The three main components of the local hippocampal response showed characteristic changes at different cortical layers.

With increasing depths the initial spike increased in magnitude and attained a maximum amplitude at 0.4-0.5 mm (Fig. 5 *A*), corresponding to the pyramidal layer. Below this stratum the amplitude rapidly decreased, and the polarity reversed at about 0.7 mm. In some other experiments the initial spike remained negative down to 1.0 mm (Fig. 5 *B*). The change at 0.5-0.6 mm from a purely negative spike to a diphasic positive/negative one suggests that the action potential is propagated along the apical dendrites away from the cell body.

The slow negative wave has its maximum at the ventricular surface and reversed already at 0.3-0.4 mm, *i.e.* above the reversal point of the initial spike and above the pyramidal cell bodies. Below the point of reversal the slow wave was recorded with a positive polarity, often of a considerable magnitude.

The third component, the small spikes occurring superimposed upon the surface negative wave, is shown in Fig. 5 *B*. In this experiment only one spike was seen. Like the initial spike it increased in magnitude and attained a maximum at 0.4 mm, corresponding to the pyramidal layer. Below this level the late spike rapidly turned diphasic and soon became purely positive. This was interpreted as downward propagation followed by a conduction block at 0.8 mm. At this level the initial spike was still revealed as a monophasic negative deflection. It is inferred from this experiment that the initial spike propagated further along the apical dendrites than did the late spike.

In Fig. 6 some more detailed observations concerning the problem of impulse propagation along cortical dendrites are illustrated. *A* shows the records obtained at 0.1 mm intervals from the surface down to 1.0 mm. The amplitude of the spike reached its maximum at about 0.4 mm. Below this level the spike became positive/negative and subsequently purely positive. In *B* the records obtained at 0.4, 0.6, 0.7, 0.8 and 1.0 mm are photographically superimposed. *C* shows a pyramidal neuron drawn to scale and at the appropriate level and the latency of the negativity of the initial spike plotted against the depth of the electrode. The graph demonstrates that the speed of propagation decreased progressively from 0.55 m/sec just below the cell bodies to 0.18 m/sec just before the conduction was blocked. In this experiment the spike was sometimes recorded as a double

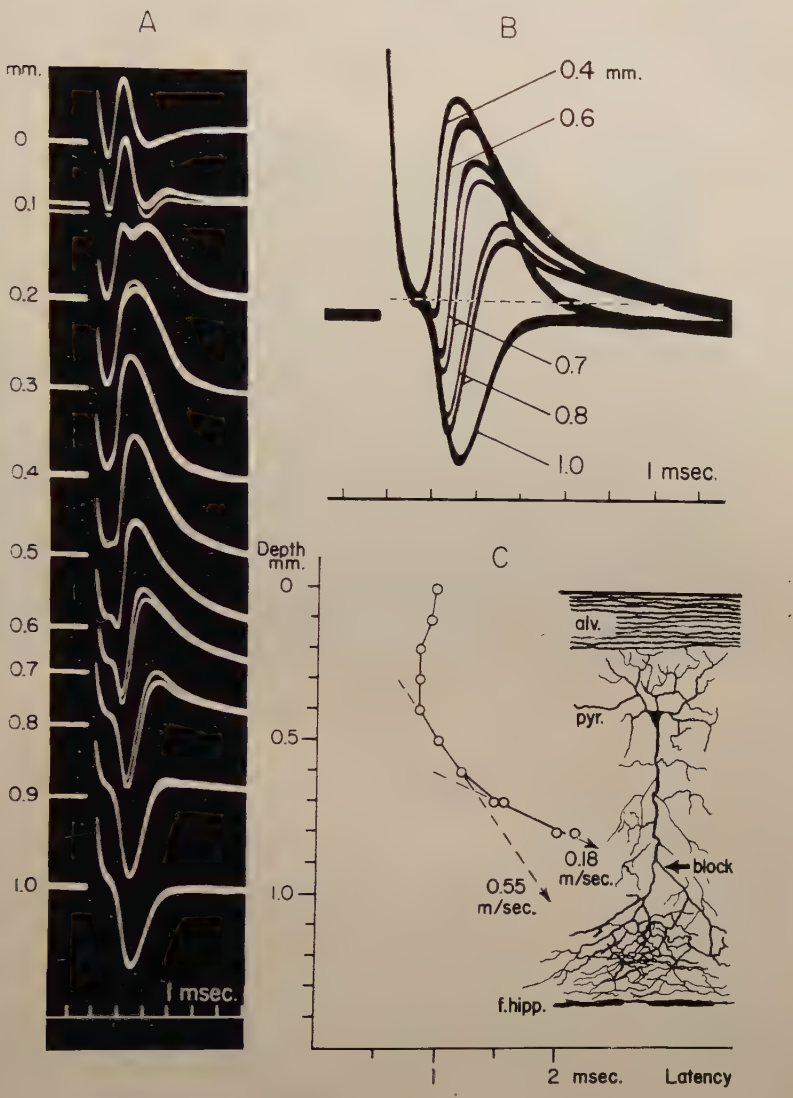


Fig. 6. – *Details of changes in latency and polarity of initial spike at different depths.*

A: responses at successive depths with 0.1 mm intervals. *B*: records from 0.4, 0.6, 0.7, 0.8 and 1.0 mm photographically superimposed. Dotted line indicates the points to which the latencies have been measured. *C*: plot of the latency of initial spike against distance from the alvear surface. To the right a pyramidal neuron drawn to scale at the correct level.

deflection. The two spikes then fused when the recording electrode was pushed down to the pyramidal layer. They had the same excitability properties and the same stimulation threshold.

Response to paired shocks. — The initial spike was not affected by a preceding supramaximal shock until the delay was about 7 msec. At shorter delays the spike was progressively diminished and at about 1.5 msec (1.0-1.7 msec.) it was totally abolished (Fig. 7 *C*). The subsequent negative wave showed a different excitability cycle.

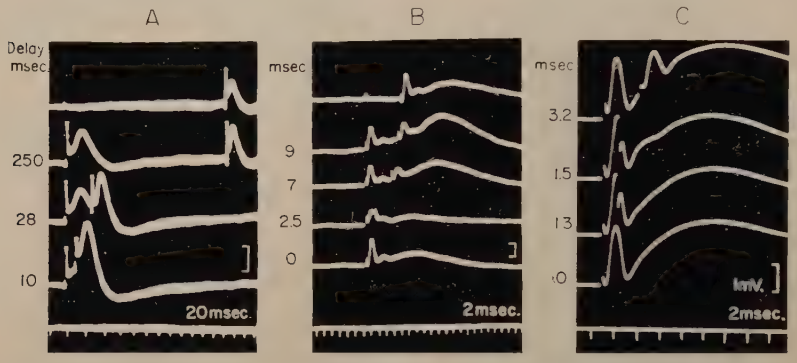


Fig. 7. – *Responses to paired shocks at different intervals.* *A*, *B* and *C*, different sweep speeds.

When supramaximal shocks were employed, this component was augmented at delays up to 300 msec, and was observed to summate to the corresponding wave of the conditioning response (Fig. 7 *A* and *B*).

Response to repetitive stimulation. — This procedure was of special value in the study of the local hippocampal response. Some pertinent observations are displayed in Fig. 8. Column *A* shows the marked facilitatory effect on the negative wave. The initial spike was not influenced. Following the repetitive stimulation the responses to single shocks demonstrated a period of post-tetanic potentiation, again affecting the negative wave and leaving the initial spike unchanged (Fig. 8 *A*, 3" and 12"). The post-tetanic potentiation lasted from some seconds to minutes according to the duration and the frequency of the stimulation.

Another example is shown in *B*. The sharp initial deflection of the local response represents the initial spike. The following slow wave, positive in this experiment, was greatly enhanced both during and after the repetitive stimulation. By the use of relatively high

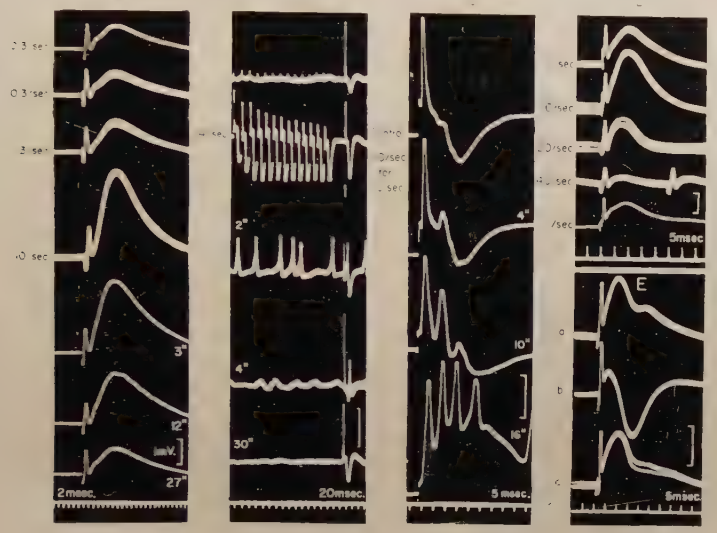


Fig. 8. – *Effect of repetitive stimulation.*

A: tetanic and post-tetanic enhancement of slow negative wave. Frequency of stimulation to the left of the four upper records. The following three records are single shock responses (0.3/sec) after a 10/sec conditioning tetanus of 10 sec duration. Numerals indicate time in secs after the end of conditioning tetanus. *B*: development of afterdiscarge after a 24 sec tetanic stimulation and its effect on the local hippocampal response. Time in secs after the end of tetanic stimulation above each of the following records. Further explanation in the text. *C*: post-tetanic enhancement of early part of slow wave and development of spike discharges. Time in secs after end of conditioning tetanus above each of the following single shock test responses. *D*: depression of slow negative wave by repetitive stimulation at different frequencies indicated to the left of each record. *E*: partial reversal of slow negative wave by repetitive stimulation. *a*: control response (0.3/ sec). *b*: 1 min after 100/ sec stimulation for 10 sec. *c*: 2 min after tetanic stimulation.

repetition rates, as in the example illustrated, sustained electrical afterdischarges occurred quite frequently. The three lowest records in *B* were obtained during and after such an event. The record marked *2"* is from the start of the afterdischarge. The spontaneous discharges are seen and in addition the enhanced initial spike of the local response. In the following record (4") the spontaneous

discharges are of small amplitude, but the initial spike is still augmented. In addition, a new spike is formed in the trough of the following positive wave. In this case the afterdischarge was of short duration. As a rule such discharges lasted for considerably longer periods. During such conditions the local hippocampal response was depressed, at first the negative wave, but shortly afterwards also the initial spike. The two components reappeared in the reverse order.

Fig. 8 *C* demonstrates a third type of effects due to repetitive stimulation. On increasing the stimulus frequency the small negative notch on the slow wave increased and developed to a negative spike. Subsequently more spikes were elicited, all being superimposed on a long-lasting negative wave. Such results were only seen in periods of increased cortical excitability.

Fig. 8 *D* and *E* shows depression or reversal of the negative wave as the result of tetanic stimulation of higher frequency (in *D* 40/sec and in *E* 100/sec). The initial spike undergoes much smaller changes. Usually the effect was changed from a facilitatory to an inhibitory one on increasing the stimulus frequency.

Effect of anoxia. — Deprivation of the oxygen supply had a differential effect on the initial spike and the negative wave (Fig. 9 *A*). The negative wave was totally depressed in the course of 3 minutes, while the initial spike at the same time showed a small increase. Following readmission of the oxygen the reappearance of the slow wave started with the development of a negative-positive wave. This component showed a great post-anoxic enhancement (2 min), but at 4 minutes the pre-anoxic value was resumed. No corresponding variation of the amplitude of the initial spike was noted. In some experiments the anoxia rapidly changed the negative wave to a diphasic negative-positive deflection which resisted anoxia for at least 5 minutes. Again the initial spike was little affected by anoxia during the same period.

From these experiments it may be concluded that the local hippocampal response consists of three parts, the initial spike, the initial part of the negative wave, and the subsequent major part of this wave. These components resist anoxia in the same order, the latter being the most susceptible one.

Effects of strychnine. — Local application of a 1 per cent solution of strychnine sulfate at the recording site markedly changed

the local hippocampal response (Fig. 9 *B*). The initial spike was not significantly affected. However, the negative wave was greatly increased both in magnitude and duration, and several negative spikes appeared superimposed upon the negative wave. Washing of the cortex with a warm Ringer's solution reversed the effect.

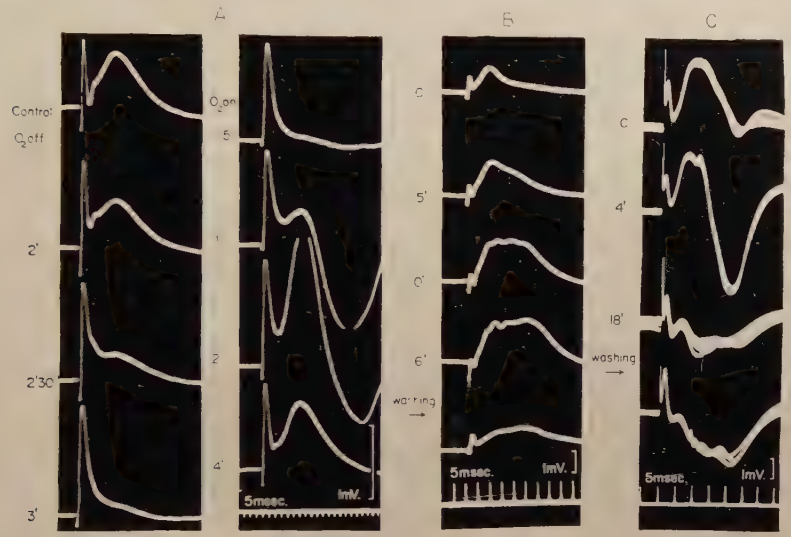


Fig. 9. – *A*: effect of anoxia. Left column development of anoxia, right column restitution. Further explanation in text. *B*: effect of strychnine. 1 per cent strychnine sulfate applied locally to the hippocampal surface close to recording electrode. *C*: effect of sodium pentobarbital (Nembutal) applied locally. Top record control. Time in min. after drug application to left of each record in *B* and *C*.

Effect of sodium pentobarbital (Nembutal). — Locally applied pentobarbital to the recording site depressed the late part of the negative wave, thereby unmasking a surface positive wave of great amplitude (Fig. 9 *C*). Later this wave was blocked, leaving only the initial spike and the first part of the negative wave almost unchanged. These results suggest that the later parts of the slow wave are due to polysynaptic activity.

Discussion

1. *Stimulus thresholds of different cortical elements.* — For the interpretation of the response obtained it is important to reveal the elements within the hippocampal cortex which have the lowest

threshold for a direct electrical stimulus. The stimulus threshold was found to be lowest in or near the pyramidal layer, suggesting that the pyramidal cell bodies, or perhaps their initial axonal segments (10, 21) are the most easily excitable elements in this region. This assumption is in agreement with observations regarding the commissural impulses between the two hippocampi (3). Stronger shocks will probably also excite a variety of other structures, including the alvear fibres.

Of special interest is the rather high stimulus threshold when the electrode was located 1.0 and 1.5 mm deep to the alveus, *i.e.* among the thinnest branches of the CA1 apical dendrites. Chang (15) tentatively attributed the local cortical response to the direct stimulation of the horizontally coursing, thin branches of the apical dendrites. This interpretation has not been generally accepted (17, 26), and the present observation of a high threshold for electrical stimulation among the thin dendritic branches supports this divergent opinion. Apical dendrites have even been claimed to be electrically inexcitable (27).

2. *Interpretation of the local hippocampal response.* — The observations presented indicate that the local hippocampal response may be divided in at least three components, differing in physiological properties.

i) *The initial spike* is ascribed to the synchronous firing of a group of pyramidal cells. As it was purely negative and of minimal latency in the pyramidal layer it is believed to be initiated in or near these cell bodies. The question arises whether the spike represents a synaptic or an antidromic activation of the pyramidal cells. The present observations do not permit a final conclusion on this point. No early activity was observed which might be interpreted as a postsynaptic potential initiating the spike. Furthermore, the spike displayed an unusually high resistance to anoxia and Nembutal and was not influenced by strychnine. All these observations would support the hypothesis of an antidromic mode of activation. On the other hand, the distribution of responses following stimulation of the alvear surface (Fig. 2), and the reduction in threshold as the stimulating electrode was pushed towards the pyramidal layer (Fig. 4) are hardly compatible with an antidromic activation as the great majority of the pyramidal cells are known to send their axons anteriorly toward the fimbria. The remarkable amplitude variations of the initial spike during afterdischarges

(Fig. 8 *B*) are similarly believed to favour the notion of a synapse in the pathway mediating the initial spike. Thus, the tentative conclusion is reached that the initial spike represents the postsynaptic firing of pyramidal cells activated by synapses close to their cell bodies. The descending collaterals of alvear fibres (13) is a possible anatomical substratum for the postulated pathway. In this context it is of interest to note that von Euler and Green (18) in their study of single units in the hippocampal field CA1 found that the cells were not invaded by antidromic impulses.

The presence of a double spike is assumed to be caused by an asynchronous activation of the pyramidal cells.

The absolute refractory period of the initial spike measured about 1.5 msec. This value is comparable with the data given for other neurons (7, 11, 23, 28). However, the value is much smaller than that obtained by Andersen (4) on commissural activation of CA1 neurons. These cells seem to have a considerably longer absolute refractory period on apical dendritical activation (as the commissural) than on activation of the cell body (as by local stimulation). Another explanation is that the commissural activation is polysynaptic. However, both histological (8) and physiological data (4) suggest that a substantial part of the commissural activation of the CA1 pyramidal neurons is monosynaptic.

The resistance of the initial spike to anoxia, strychnine and nembutal indicates that the synaptic transmission in question probably is monosynaptic.

ii) *The negative wave* is regarded as being composed of two different parts. The first part is fairly resistant while the following, major part of this wave was markedly influenced by repetitive stimulation, paired shocks, anoxia, strychnine and nembutal. Both parts of the negative wave were recorded with reversed polarity below 0.3-0.4 mm from the ventricular surface. Therefore, they are both ascribed to activity taking place in the layer of the basal dendrites, the stratum oriens. The last, sensitive component is thought to be due to polysynaptic activation of pyramidal cells, perhaps by the way of the basket cells of Lorente de Nó (24). The interpretation of the first part is more difficult. Occasionally it has been observed to increase, giving rise to a spike (Fig. 7 *C*) and may accordingly represent an excitatory postsynaptic potential elicited at the basal dendrites. However, the present data do not permit a definite interpretation of this component.

iii) *The late spikes* are assumed to represent discharges of polysynaptically activated pyramidal cells. This interpretation is based upon their high threshold, their appearance following repetitive stimulation or application of strychnine, and their early disappearance in periods of depressed excitability. Further, they are recorded with the greatest magnitude in the pyramidal layer.

3. *Impulse propagation in dendrites.* — In spite of the flourishing literature on this subject, general agreement on this point is still lacking. Purpura and Grundfest (27) deny the possibility of electrical excitability and thereby propagation of spikes within dendrites. A second group, especially Bishop and coworkers, states that impulses may spread electrotonically in a somatofugal direction in the cortical apical dendrites (5, 6, 14). On the other hand, Cragg and Hamlyn (16) and Andersen (4), studying the CA1 dendrites, have obtained results suggesting propagation along these structures. Some of the controversy may possibly be explained by regional differences in the dendritic arrangement and in their physiological properties in various parts of the central nervous system.

The present study has given evidence for a somatofugal propagation of impulses along the apical dendrites of the CA1 neurons. The initial spike was recorded diphasic positive/negative below the pyramidal layer (Fig. 5 *A*, 6). As the record obtained from 0.8 mm below the surface (Fig. 6) shows persisting negativity at an instant where the negativity of the 0.4 mm record has vanished (4 msec after the onset of the stimulus), the observed spike-propagation can not solely be explained as due to electrotonic spread. Freygang (20) has calculated that an electrotonic potential spreading along a dendrite of 5 μ diameter will display a latency of the order of 0.1-0.2 msec at a distance of 0.5 mm from the origin of the electrotonus. The shafts of the CA1 apical dendrites have diameters from 2-8 μ, thus similar to the model of Freygang, but the increase of latency of the initial spike over a distance of 0.5 mm was about 1.5 msec, *i.e.* much greater than may be explained by electrotonic spread.

The speed of propagation is remarkably consistent with that given for the same dendrites by Cragg and Hamly (16), using intracortical stimulation, and by Andersen (4), studying their synaptic activation. In the latter investigation the spike was found to have its shortest latency at the apical dendritic shafts and was propagated towards the soma. In the present study, local stimulation excited the spike at the soma and the latency increased along the apical

dendrites. These results suggest that the spike may be produced in different parts of the neurone, and that the conduction along the dendrites is not only due to electrotonic spread. The apical dendritic shafts of the pyramidal cells are by far the most likely elements to mediate the propagated spike.

The monosynaptic evoked initial spike seems to be propagated further along the apical dendrites than does the polysynaptic activated late spikes (Fig. 5 *B*). The latter also shows a diphasicity which may be regarded as a sign of somatofugal propagation, but conduction block — indicated by pure positivity — occurs at a level at which the initial spike is still negative. The extent to which an impulse may invade the apical dendrites is possibly related to the effectiveness of the soma activation. This view is in agreement with the observations of von Euler *et al.* (19) who where able to force the impulses down in the apical dendrites of the hippocampus only by repetitive stimulation of the dorsal fornix. Single shock stimulation was not sufficiently effective to make the impulses invade the dendritic tree.

4. *Relation to the local neocortical potential.* — The local hippocampal response is markedly different from the local neocortical potential. No initial spike, and only occasionally late spikes have recorded been in the latter (15, 25). However, Brooks and Enger (12) attributed their " fast response " to synaptic activation of pyramidal cells by way of horizontally coursing axons.

The surface negative wave of the hippocampal response has many properties in common with the local neocortical response. These are the surface negativity, ability of summation, small or lacking refractory period, enhancement by strychnine, and a moderate susceptibility to anoxia and pentobarbital. It does, therefore, not seem unlikely that at least the first part of the negative wave of the local hippocampal response corresponds to the surface negative wave of the local neocortical potential.

5. *Relation to the local cerebellar potential.* — The two local responses have some similarity in the form (22). Both exhibit a sharp spike, attributable to the discharge of the dominating cortical elements, *i. e.* Purkinje cells and pyramidal cells, and a slow surface negative wave which is interpreted as an excitatory postsynaptic potential initiated at the dendrites lying near the surface. However, definite differences exist. Thus, post-tetanic potentiation was absent in the cerebellar response while it was a prominent phenomenon in

the hippocampus. Strychnine enhanced parts of the hippocampal response enormously, but did not affect the cerebellar potential. These observations probably reflect a greater amount of polysynaptic circuits put into action in the hippocampal cortex compared with the cerebellum. The greater ease with which long neuron chains may be activated in the hippocampus is in accord with the frequent occurrence of afterdischarges in this cortical area.

Summary

1. The local response of the field CA1 of the hippocampus has been studied in rabbits under urethane-chloralose anesthesia. The response consisted of an initial negative spike (1-3 msec) followed by a slower surface negative wave (25-60 msec), sometimes with one or more negative spikes superimposed.

2. The response is assumed to be evoked by the direct electrical stimulation of pyramidal cell bodies or the initial part of their axons and apical dendrites. It was recorded from a rather small, wedge shaped zone extending from the stimulated point rostrally and somewhat laterally towards the fimbria. The maximal distribution of the negative wave was a little lateral to that of the initial spike. The conduction velocity of the latter was 1.3-3.3 m/sec.

3. The initial spike is regarded as the postsynaptic discharge of the pyramidal cells. The activation takes place at the level of the cell body and is propagated into the apical dendritic shaft with a velocity of about 0.5 msec, decreasing to about 0.2 msec just before the site of the conduction block. The terminal arborizations of the apical dendrites were apparently not invaded.

4. The surface negative wave reversed its polarity just above the pyramidal layer. It is partly attributed to the synaptic activation of the basal dendrites. If this activation becomes sufficiently intense, late spikes occur as the sign of polysynaptic initiated pyramidal cell discharges. These secondarily activated pyramidal cell discharges are also propagated into the apical dendrites, but are blocked closer to the cell body than is the monosynaptically activated initial spike.

5. The results are discussed in relation to the local responses of the neocortex and the cerebellar cortex.

Acknowledgments

The authors wish to express their gratitude to Professor Birger R. Kaada for valuable advice and for placing the facilities of the laboratory at their disposal. The assistance of our electroengineer G. Lilleheil is gratefully acknowledged.

The present work has been sponsored in part by the Air Force Office of Scientific Research of the Air Research and Development Command, United States Air Force, through its European Office, under Contract No AF 61(514)-1127, and in part by the Norwegian Research Council for Science and the Humanities.

Addendum

During the preparation of the manuscript another work appeared, dealing with the local cortical response of the hippocampus in rabbits (Kandel E. R., Spencer W. A. and Brinley F. J. Jr. Transient and long-lasting electrical responses to direct hippocampal stimulation. *Amer. J. Physiol., 198*: 687-692, 1960). This investigation deals with properties of the slow negative wave and its reaction to topical application of GABA. Contrary to the findings by the present authors, Kandel *et al.* only occasionally observed the initial spike and have not investigated the properties of this component of the local hippocampal response.

REFERENCES

1. Adrian, E. D. The spread of activity in the cerebral cortex. *J. Physiol., 88*: 127-161, 1936.
2. Andersen, P. Localization of micro-electrode sites by silver impregnation. *Acta physiol. scand., 35*: 305-311, 1956.
3. Andersen, P. Interhippocampal impulses. I. Origin, course and distribution in cat, rabbit and rat. *Acta physiol. scand., 47*: 63-90, 1959.
4. Andersen, P. Interhippocampal impulses. II. Apical dendritic activation of CA1 neurons. *Acta physiol. scand., 48*: 178-208, 1960.
5. Bishop, G. H. and Clare, M. H. Sites of origin of electric potentials in striate cortex. *J. Neurophysiol., 15*: 201-220, 1952.
6. Bishop, G. H. and Clare, M. H. Responses of cortex to direct electrical stimuli applied at different depths. *J. Neurophysiol., 16*: 1-19, 1953.
7. Bishop, P. O. and Evans, W. A. The refractory period of the sensory synapses of the lateral geniculate nucleus. *J. Physiol., 134*: 538-557, 1956.
8. Blackstad, T. Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. *J. comp. Neurol., 105*: 417-537, 1956.
9. Bremer, F. Cerebral and cerebellar potentials. *Physiol. Rev., 38*: 357-388, 1958.
10. Brock, L. G., Coombs, J. S. and Eccles, J. C. Intracellular recording from antidromically activated motoneurons. *J. Physiol., 122*: 429-461, 1953.
11. Brooks, C. McC., Downman, C. B. B. and Eccles, J. C. After-potentials and excitability of spinal motoneurons following antidromic activation. *J. Neurophysiol., 13*: 9-38, 1950.
12. Brooks, V. B. and Enger, P. S. Spread of directly evoked responses in the cat's cerebral cortex. *J. gen. Physiol., 42*: 761-777, 1959.
13. Cajal, S. R. *Histologie du système nerveux de l'homme et des vertebrés* Paris, A. Maloine, vol. 2, 993 pp., 1911.
14. Clare, M. H. and Bishop, G. H. Properties of dendrites; apical dendrites of the cat cortex. *EEG. clin. Neurophysiol., 7*: 85-98, 1955.

15. CHANG, H.-T. Dendritic potential of cortical neurons produced by direct electrical stimulation of the cerebral cortex. *J. Neurophysiol.*, *14*: 1-21, 1951.
16. CRAGG, B. G. and HAMLYN, L. H. Action potentials of the pyramidal neurones in the hippocampus of the rabbit. *J. Physiol.*, *129*: 608-627, 1955.
17. ECCLES, J. C. Interpretation of action potentials evoked in the cerebral cortex. *EEG. clin. Neurophysiol.*, *3*: 449-463, 1951.
18. EULER, C. von and GREEN, J. D. Excitation, inhibition and rhythmical activity in hippocampal pyramidal cells in rabbit. *Acta physiol. scand.*, *48*: 110-125, 1960.
19. EULER, C. von, GREEN, J. D. and RICCI, G. The role of hippocampal dendrites in evoked responses and after-discharges. *Acta physiol. scand.*, *42*: 87-111, 1958.
20. FREYGANG, W. H. Jr. An analysis of extracellular potentials from single neurons in the lateral geniculate nucleus of the cat. *J. gen. Physiol.*, *41*: 543-564, 1958.
21. FUORTES, M. G. F., FRANK, K. and BECKER, M. C. Steps in the production of motoneuron spikes. *J. gen. Physiol.*, *40*: 735-752, 1957.
22. JANSEN, J. Jr. and ANDERSEN, P. Cerebellar cortical response to local electrical stimulation. 1961. In preparation.
23. LLOYD, D. P. C. The interaction of antidromic and orthodromic volleys in a segmental spinal motor nucleus. *J. Neurophysiol.*, *6*: 143-151, 1943.
24. LORENTE DE NÓ, R. Studies on the structure of the cerebral cortex. II. Continuation of the study of the Ammonic system. *J. Psychol. Neurol., Lpz.*, *46*: 113-177, 1934.
25. OCHS, S. The direct cortical response. *J. Neurophysiol.*, *19*: 513-523, 1956.
26. PURPURA, D. P. Nature of electrocortical potentials and synaptic organization in cerebral and cerebellar cortex. *Int. Rev. Neurobiol.*, *1*: 47-163, 1959.
27. PURPURA, D. P. and GRUNDEST, H. Nature of dendritic potentials and synaptic mechanisms in cerebral cortex of cat. *J. Neurophysiol.*, *19*: 573-595, 1956.
28. THERMAN, P. O. Transmission of impulses through the Burdach nucleus. *J. Neurophysiol.*, *4*: 153-166, 1941.

CEREBRAL CONTROL OF TRANSMISSION TO THE VENTRAL SPINO-CEREBELLAR TRACT

F. MAGNI [1] AND O. OSCARSSON [2]
Department of Physiology, Australian National University, Canberra, Australia

Introduction

The functional organization of the ventral spino-cerebellar tract (VSCT) has been studied in considerable detail (9, 32, 34, 36). The VSCT neurones are located in the lateral part of the intermediate zone and adjacent parts of the dorsal and ventral horns, and send their axons up the contralateral ventral quadrant of the spinal cord (20). They are monosynaptically activated by ipsilateral I*b* (Golgi tendon organ) afferents, and polysynaptically inhibited from the flexor reflex afferents, *i.e.* group II and III muscle afferents, skin and high threshold joint afferents (9, 32, 34, 10, 18). In the decerebrate preparation the inhibition is strongly suppressed because of a tonically active control system described by Holmqvist, Lundberg and Oscarsson (18). This system originates from cells in the lower brain stem and descends in the dorsolateral funiculus of the spinal cord. From each side a bilateral control is exerted.

Experiments suggesting a different supraspinal control system were recently reported (36): repetitive stimulation of the dissected dorsolateral funiculus was followed by a strong inhibition of ipsilateral VSCT neurones. It has now been shown that this inhibition can be caused by stimulation of pyramidal tract fibres coming from the sensorimotor cortex. In addition, there has been discovered an extrapyramidal control system which originates in the cerebral cortex and exerts a powerful excitatory action on the VSCT neurones.

---

[1] Present address: Institute of Physiology, University of Pisa, Italy.
[2] Present address: Institute of Physiology, University of Lund, Sweden.

Methods

The experiments were performed on 20 cats under light pentobarbitone anaesthesia. The hamstring, triceps, and sural nerves were dissected and mounted on stimulating electrodes. The nerves were usually prepared bilaterally. The L_1 and L_2 spinal segments were exposed. A horizontal section reaching to the midline of the spinal cord and going through the level of the denticulate ligaments was made from the upper part of the L_1 segment down to the rostral border of the insertion of the L_2 roots. The ventral quadrant of the cord was transected at the rostral end of this section; a slight lateral pull sufficed to separate it from the midline. The ventral quadrant was mounted on recording electrodes, one placed near the severed end, the other, close to the place where the quadrant was in continuity with the intact spinal cord at mid-L_2. In most experiments the ventral quadrant was prepared bilaterally.

In some experiments intra-axonal recording was made from fibres in the ventral quadrant at the lower part of the L_2 segment. The technique was similar to that described by Laporte, Lundberg and Oscarsson (26). In a few experiments the dorsolateral funiculus (= dorsal half of lateral funiculus) on one side was dissected at Th8 after a laminectomy including a few thoracic vertebrate. In these cases the dorsal funiculus of the same side was severed about 5 mm caudally of the dissected dorsolateral funiculus. The latter was mounted on stimulating electrodes. The frontal lobe and rostral part of the parietal lobe was exposed, as a rule, bilaterally. Pools of mineral oil covering the dissected nerves, cortex, and exposed segment of the spinal cord were kept at a temperature of about 38° C.

In some experiments designed for making lesions in the pyramids the ventral aspect of the medulla oblongata was exposed. The lesions were controlled histologically by serial sections 25 μ thick.

Cortical stimulation was performed with a silver ball electrode (anode) touching the cortex, the indifferent electrode being placed in the temporal muscle, or as a ring around the exposed cortex. The stimuli were condensor discharges with a half decay of about 40 microsec. Usually repetitive stimulation at a frequency of 660 per second (1-16 stimuli) was employed. The threshold for any appreciable effect on the VSCT was usually about 4 V.

Conventional amplifying and recording technique was used. The records were usually obtained by photographic superposition of 10 traces in order to reject random noise.

Results

1. *Cortical effects on ascending tracts in the ventral quadrant.* — The spike-like, monosynaptic VSCT mass discharge elicited by stimulation of group I muscle afferents and recorded from the dissected contralateral ventral quadrant, was used for testing inhibitory and facilitatory effects from cortex. In order to obtain a large test discharge combined stimulation of the hamstring and triceps nerves was usually employed. In Fig. 1, the VSCT discharge was recorded bilaterally, the unconditioned discharges being shown to the left. Points *A-D* of the right cortex (left diagram) were stimulated repetitively and the effects on the right (upper row of records) and left (centre row) VSCT discharges are shown in the appropriately labelled

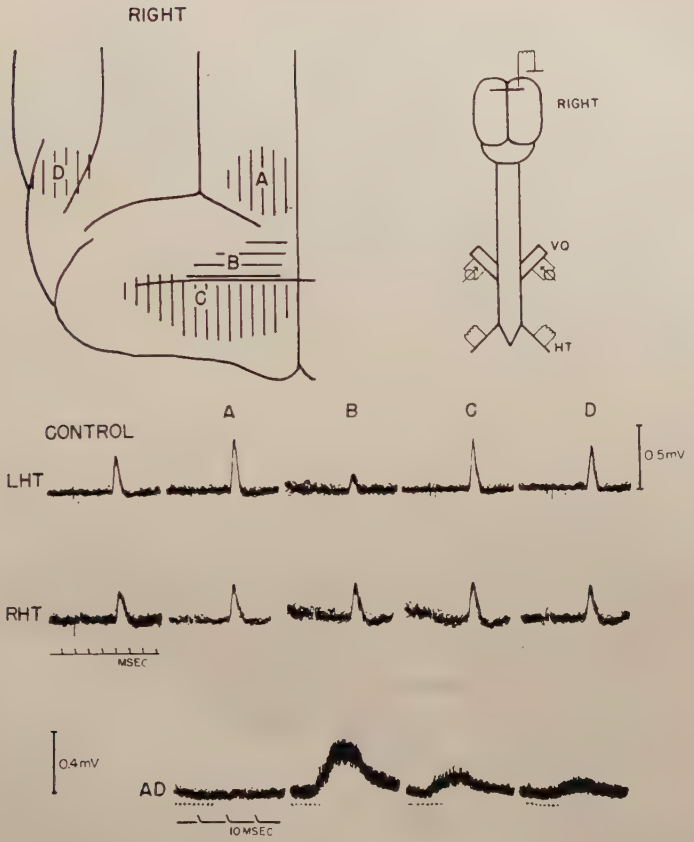


Fig. 1. – *Effects produced by stimulation of the cerebral cortex on certain ascending tracts.*

The stimulating and recording arrangement is shown by the diagrams. The ventral quadrants (VQ's) of the spinal cord were recorded from at the L_2 segment. Upper row of records shows the VSCT discharge in the right VQ elicited by combined stimulation of the left hamstring and triceps nerves (LHT). Middle row shows the VSCT discharge in the left VQ elicited by combined stimulation of the right hamstring and triceps nerves (RHT). The records in the left vertical column show the unconditioned VSCT discharges, the columns labelled *A-D* show the VSCT discharges conditioned by repetitive cortical stimulation (preceding the sweep) in points appropriately marked on left diagram (anterior part of right hemispere, viewed from above). The number of stimuli was 15, the frequency 660 per second. The interval between the first conditioning stimulus and the VSCT discharge was 30 msec in upper row, and 25 msec in middle row of records. Lower row of records shows the ascending discharge (*AD*) evoked by cortical stimulation alone. Note absence of discharge on stimulation of point *A*, and long latency of discharge elicited from point *D*. The amplification was the same for the two lower rows of record. All the records were formed by superposition of 10-15 faint traces.

columns of records. Strong effects were observed with the right VSCT response. Stimulation of points *A*, *C* and *D* resulted in facilitation and stimulation of point *B* caused inhibition. Similar results were obtained in other experiments and the cortical areas responsible for facilitation and inhibition are hatched in the left diagram. Vertical hatching indicates the facilitatory areas and horizontal hatching the inhibitory area. Weak facilitation of the left VSCT discharge was observed from all points (centre row of records).

Repetitive stimulation of points *B*, *C* and *D* elicited an ascending discharge in the ventral quadrant (lower row of records). This discharge was evoked bilaterally, there being no significant difference in size between the discharges in the two quadrants (Fig. 2 *K*, *L*; Fig. 3 *G*, *H*).

Further information about the nature of the cortical effects was obtained by intra-axonal recording from fibres in the ventral quadrant. It has previously been shown (35) that the majority of the coarse ascending fibres in this area of the spinal cord belong to two groups: i) VSCT fibres monosynaptically activated from contralateral group I afferents. ii) Fibres ascending to the brain stem and polysynaptically activated from ipsilateral and contralateral flexor reflex afferents; the tract they constitute will be denoted VFRT. Typical records from these two types of fibres are shown in Fig. 2. The upper traces record intra-axonal potentials and the lower traces, mass discharges in the ventral quadrant. Records *A*-*F* show a VSCT unit monosynaptically activated from the hamstring nerve (*A*) (cf. 34). In record *B*, the hamstring nerve was stimulated with a strength slightly submaximal for group I afferents. Both the unitary spike and the mass discharge were inhibited either by a conditioning stimulus applied to the sural nerve (*C*) or by repetitive stimulation of the inhibitory cortex (*D*). There was no activity in the VSCT unit corresponding to the ascending mass discharge evoked by cortical stimulation (*D*), hence the inhibition of the VSCT response was genuine and not due to occlusion. Similar findings were made with the other VSCT units recorded from. Record *E* was obtained on slightly weaker stimulation of the hamstring nerve. The unitary spike appeared only occasionally as shown by the superposed traces. Stimulation of the facilitatory area increased the VSCT mass discharge and made the unitary spike appear regularly (*F*). In our experiments the cortical facilitation was never strong enough to evoke spikes in the VSCT units; it was only revealed as a subliminal excitation by the method of monosynaptic testing.

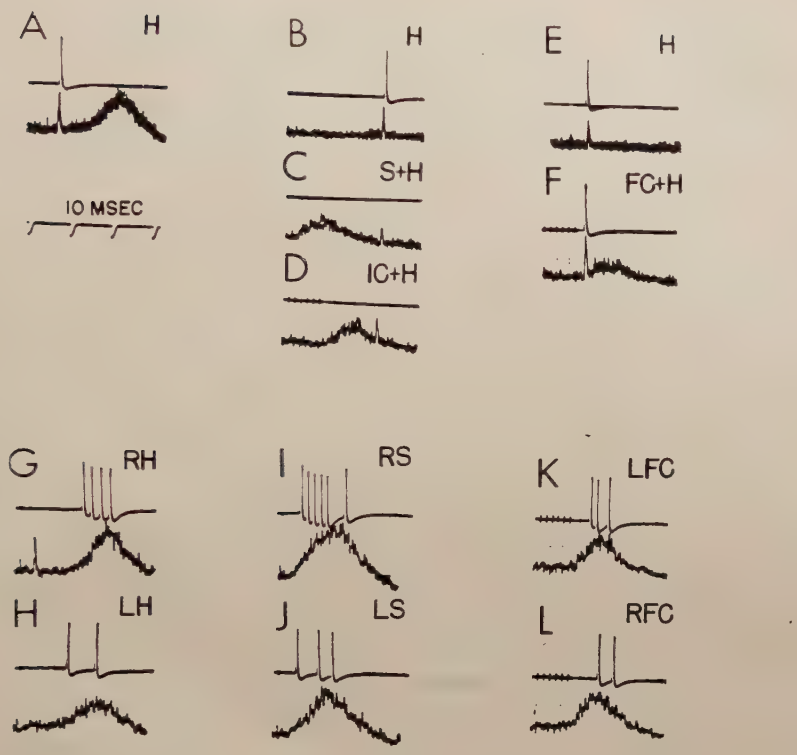


Fig. 2. – *Effect of cortical stimulation on VSCT and VFRT units.*

The stimulating and recording arrangement as in Fig. 1, but only the right ventral quadrant was recorded from. In addition, intraaxonal recording was performed from fibres in the right ventral quadrant at the L_2-L_3 boundary. The right and left hamstring (*H*) and sural (*S*) nerves were mounted for stimulation. Simultaneous recording from fibre (upper traces) and from ventral quadrant (lower traces). *Records A-F* from a VSCT unit monosynaptically activated by group I afferents in the left hamstring nerve. All records were formed by superposition of about 10 faint traces. *A*, supramaximal stimulation of the left hamstring nerve. *B-D*, stimulation of the left hamstring nerve at a slightly submaximal group I strength which unconditioned regularly elicited a spike in the unit (*B*). *C*, inhibition of spike and VSCT mass discharge produced by a single conditioning volley in the left sural nerve. *D*, inhibition produced by repetitive stimulation (six stimuli) of the right inhibitory cortex (IC). *E-F*, stimulation of the left hamstring nerve at a submaximal group I strength which unconditioned only sometimes evoked a spike in the unit (*E*). *F*, conditioning by repetitive stimulation (six stimuli) of the right rostral facilitatory area (FC) resulted in facilitation; the spike appeared regularly. *Records G-L* from a VFRT unit activated by skin and high threshold muscle afferents in all tested nerves (*G-J*, right and left hamstring and sural nerves). This unit was also activated by repetitive stimulation (six stimuli) of the left and right cortex of the anterior sygmoid gyrus (LFC, RFC). Note similarity in time course of mass discharge and unit activity. Same sweep speed in all records (*A-L*).

Records *G-L* were obtained from a VFRT fibre. This fibre was typically activated from right and left muscle and skin nerves (*G-J*) and also by stimulation of either left or right cortex (*K*, *L*). The VFRT fibres were responsible for the late discharges recorded in the ventral quadrant on supramaximal stimulation of various nerves (cf. 35) and also for the similar discharge which appeared on cortical stimulation.

2. *Descending pathways.* — Experiments as that illustrated in Fig. 3 were performed in order to decide if the pyramidal tract mediated any of the effects described in the preceding section. The right VSCT discharge was inhibited from the right inhibitory cortex (RIC) (*C*) and facilitated from the rostral right facilitatory cortex

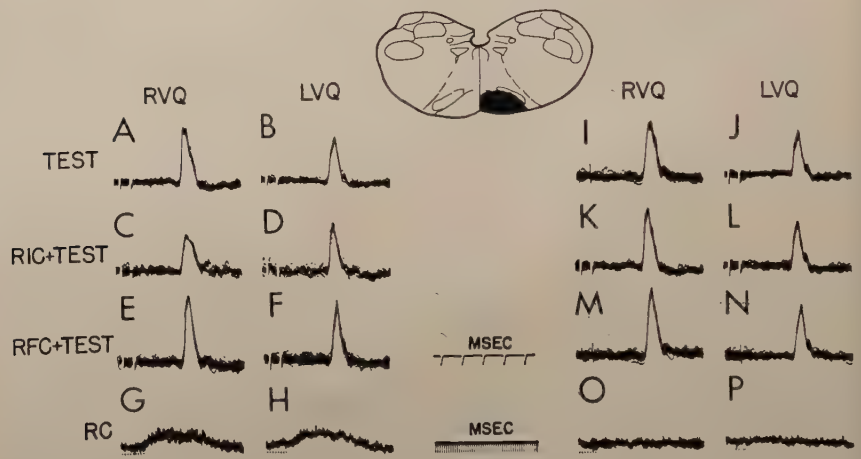


Fig. 3. – *The pyramidal tract as pathway for cortical inhibition but not for cortical facilitation.*

Stimulating and recording arrangement as in Fig. 1. The right cerebral cortex was stimulated in the inhibitory area (RIC) and the rostral facilitatory area (RFC, RC). *A-H* were obtained before, and *I-P* after, a lesion made in the right pyramid as shown by the diagram. The four vertical columns of records were obtained from the right and left ventral quadrants (RVQ and LVQ), as indicated. *A-F* and *I-N* show the VSCT discharges evoked by combined stimulation of contralateral hamstring and triceps nerves. Upper horizontal row of records shows control discharges. Row indicated RIC + TEST shows effects produced by repetitive stimulation (12 stimuli preceding the sweep) of the right inhibitory area. Note change of inhibition to facilitation after lesion (*C*, *K*). Row indicated RFC + TEST shows effects produced by repetitive stimulation (as for RIC) of right facilitatory area. Note that the facilitation remained after the lesion, though it was slightly decreased in *L* and *N*. *G* and *H* show the ascending discharges evoked from the right cortex (9 stimuli) which disappeared after the lesion (*O*, *P*). Note different time scales for *A-F*, *I-N* and for *G*, *H*, *O* and *P*.

(RFC) (*E*), whereas the left VSCT discharge was facilitated from both these cortical-areas (*D*, *F*). After right pyramidotomy the inhibition reversed to facilitation (compare *C* and *K*). The facilitation of the right VSCT remained unchanged (*E*, *M*), whereas that of the left VSCT was slightly decreased (*D*, *L* and *F*, *N*). Records *G* and *H* show the ascending discharges evoked bilaterally in the VFRT from the right cortex; following the pyramidotomy this discharge was bilaterally abolished (*O*, *P*). Similar results were obtained in other experiments; in two of them it was shown that the facilitation remained also after a bilateral pyramidotomy. In some cases the facilitation decreased slightly following the unilateral (cf. Fig. 3) or bilateral pyramidotomy; presumably there was some accidental damage of structures surrounding the pyramids. It is concluded that the cortical facilitation of the VSCT was mediated by an extrapyramidal pathway, whereas the VSCT inhibition and the ascending discharge in VFRT were induced by activity in the pyramidal tract.

The experiments illustrated in Fig. 4 were designed to decide in which part of the spinal cord the facilitatory system descended. Records *A-F* show facilitation of the right (*A-C*) and left (*D-F*) VSCT discharges. Unusually, the facilitation was as marked with the contralateral VSCT discharge (*B*, *F*) as with the ipsilateral one (*C*, *E*). After transection of the right dorsal quadrant (black in diagram) the right discharge was still facilitated (*H*, *I*), whereas the facilitation of the left discharge had disappeared (*K*, *L*). It is concluded that the facilitation from ipsilateral and contralateral cortex was exclusively mediated by fibres descending in the cord ipsilaterally to the cell bodies of the VSCT neurones.

It has previously been shown that the ventral quadrant of the spinal cord contains descending fibres which monosynaptically activate ipsilateral VSCT cells (9, 19, 34). Presumably these fibres originate from cells in the brain stem and mediate effects from the cerebellar cortex (19). We have now excluded that the present facilitatory system is identical with this ventral pathway; it could *a priori* not be excluded as part of that system might remain after dissection of the ventral quadrants. Records *M-R* were obtained from a preparation with initially intact spinal cord except for the dissected right ventral quadrant (left diagram). Upper records (*M*, *O*, *Q*) show the test VSCT discharges and lower records (*N*, *P*, *R*) these discharges facilitated from the right cortex. There was virtually

no decrease of the facilitation when the left ventral quadrant was transected (centre diagram; *O*, *P*), or when in addition the right dorsolateral funiculus and both dorsal funiculi were severed (right diagram; *Q*, *R*.)

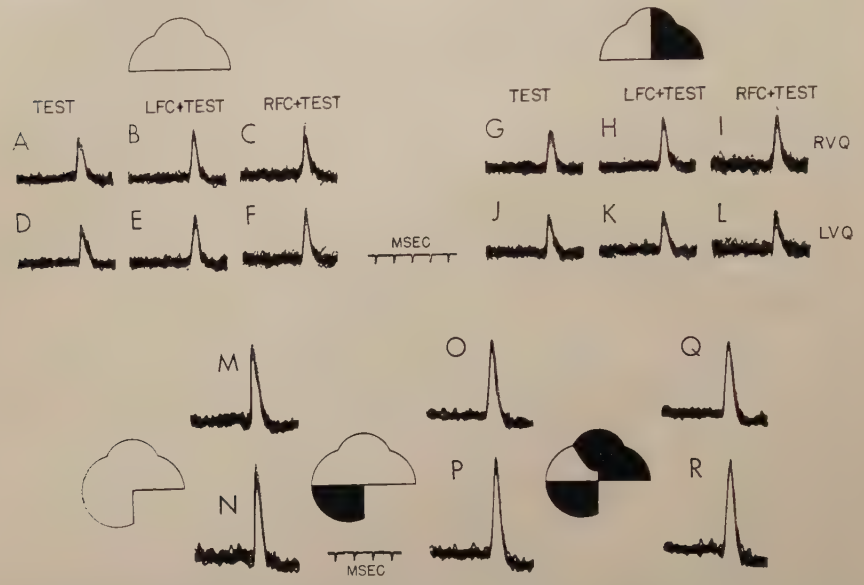


Fig. 4. – *Identification of the excitatory pathway as part of the ipsilateral (relative to the VSCT cells) dorsolateral funiculus.*

Records A-L from one experiment. Stimulating and recording arrangement as in Fig. 1, but the cortex was exposed bilaterally for stimulation. The VSCT discharge was evoked by combined stimulation of the contralateral hamstring and triceps nerves. The cortical stimulation (12 stimuli) was applied to the rostral facilitatory area (FC). *A-F* were obtained with the dorsal half of the spinal cord intact, *G-L* after transection of the right dorsal quadrant. The upper row of records shows VSCT discharges in the right ventral quadrant (RVQ), the lower row VSCT discharges in the left ventral quadrant (LVQ). *A*, *D*, *G* and *L* are unconditioned discharges. Vertical columns of records indicated LFC + TEST and RFC + TEST show the VSCT discharges conditioned by stimulation of indicated left and right cortical areas (the stimuli precede the sweep). Note that the facilitation unusually was of the same intensity from ipsilateral and contralateral cortex. Note that the facilitation of the right VSCT discharge remained after the lesion, while it was abolished in the left VSCT. Records M-R from a different experiment. The spinal cord was intact initially, except for the dissected right ventral quadrant at L_2. The VSCT discharge was evoked by combined stimulation of the left hamstring and triceps nerves and is shown unconditioned in *M*, *O* and *Q*. *N*, *P* and *R* show the VSCT discharge conditioned by repetitive stimulation (12 stimuli) of the right rostral facilitatory area. *M* and *N* before, and *O* and *P* after, transection of the left ventral quadrant (indicated by black in the diagram). *Q* and *R* after additional transection of the right dorsolateral funiculus and both dorsal funiculi (black in diagram). Note that the facilitation remained unchanged.

Similar experiments were performed with respect to the inhibitory pathway. It was found that this pathway was contained within the dorsolateral funiculus ipsilateral to the VSCT cells. This is in accordance with the fact that the pyramidal tract in the cat originating from the pericruciate cortex and reaching caudal segments of the cord, is mainly or exclusively crossed and located in the dorsolateral funiculus (2, 5, 6).

3. *Cortical areas.* — The areas from which the various cortical effects could be elicited were determined by systematic mapping. Usually 12 stimuli were used, the effect on the VSCT being tested after a fixed interval approximately corresponding to maximal inhibition as well as maximal facilitation. The stimulus strength was slightly above threshold for a barely appreciable effect on the VSCT discharge. This strength had to be approximately doubled in order to evoke a localized contraction in head or forelimb musculature (motor effects in other parts of the body had a higher threshold). This contraction ceased when the electrode was moved about 1 mm along the cortical surface; clearly the stimulus strength used for mapping did not cause any appreciable cortical spread. The cortex was explored by moving the electrode stepwise 1 mm, records being taken at each position of ipsilateral and contralateral effects on the VSCT, and of the ascending discharge in VFRT.

In Fig. 5, the inhibitory area is indicated by horizontal hatching, and, in maps *B* and *D*, diagonal hatching shows regions from which the ascending discharge could be evoked. The inhibitory area was limited to the medial part of the posterior sygmoid gyrus (map *A*); maximal inhibition was usually obtained by stimulation close to the cruciate sulcus. No part of the inhibitory area was concealed by facilitation as shown in two preparations which for unknown reasons were deficient of the facilitatory effect, whereas the pyramidal effects, inhibition to VSCT and excitation to VFRT, were of normal strength (map *D*). Inhibition was never observed from any other area of the explored cortex and it was always strictly unilateral. The ascending discharge was elicited from the medial part of the posterior sygmoid gyrus and from the lateral part of the anterior sygmoid gyrus (maps *B*, *D*). It was usually largest when evoked from points close to the cruciate sulcus, being maximal from points sometimes anterior and sometimes posterior to this sulcus. It was evoked bilaterally from the whole area. An ascending discharge was also

elicited from a region corresponding to the hindlimb area of the somatic area II (maps *B*, *D*). This discharge was small and had a relatively long latency (Fig. 1).

Both the inhibition of VSCT and the ascending discharge were due to activity in the pyramidal tract. The inhibition of VSCT neurones was tested on cells activated from hindlimb nerves which

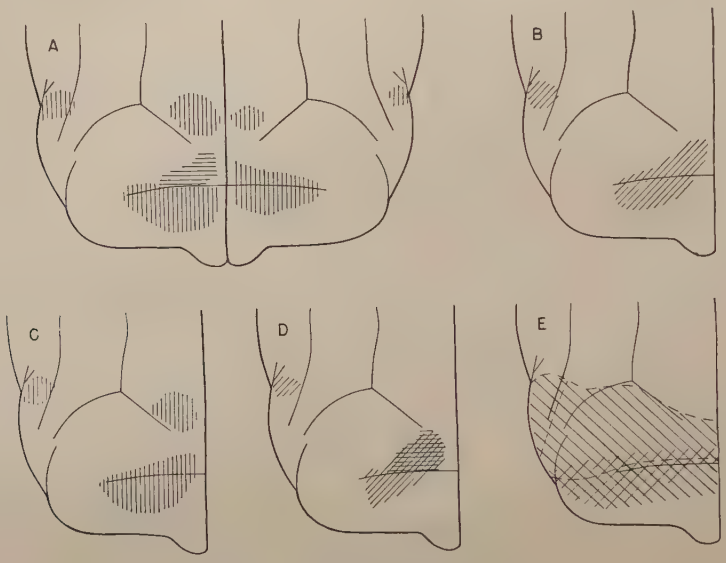


Fig. 5. – *Cortical areas influencing the VSCT and VFRT.*

Dorsal aspect of cerebrum. *A*, areas influencing the right VSCT: horizontal hatching represents inhibition, vertical, facilitation. *B*, areas producing a discharge in the right VFRT. *C*, areas influencing the VSCT after right pyramidotomy (hatching as in *A*). *D*, areas influencing VSCT and producing a discharge in VFRT, in a preparation deficient of cortical facilitation to VSCT (hatching as in *A* and *B*). *E*, distribution of antidromic pyramidal potentials (hatched and cross-hatched) according to Woolsey and Chang (41) and sensory areas I and II (surrounded by broken lines) according to Woolsey and Fairman (42). See the text.

presumably explains the medial location of the inhibitory area, corresponding to the hindlimb area of the sensorimotor cortex (cf. 1, 13, 25, 39, 42). The more lateral and anterior extension of the area responsible for the ascending discharge may correspond to the body area. This discharge was presumably evoked in units with receptive fields not only on the hindlimbs but also more rostrally on the body area.

A correlation of the present areas with either sensory or motor

cortex is of special interest. The pyramidal tract takes origin from both these cortical areas; the part originating in the sensory cortex terminates in sensory nuclei and has been suggested to control transmission to sensory pathways (22). Hatching and cross-hatching in map *E*, Fig. 5, indicates the origin of the pyramidal tract as determined by recording of antidromic potentials (23, 41, but cf. 21, 24). This area corresponds reasonably well to *area gigantopyramidalis* of Brodmann (3, cf. 4). The motor cortex has been delimited by studies of the motor effects that can be evoked on cortical stimulation. There is agreement that the motor area includes the posterior sygmoid gyrus and a lateral part of the anterior sygmoid gyrus (13, 25, 27, 39, 40). On the other hand, the somatosensory cortex is restricted to the postcruciate cortex, the sensory areas I and II being surrounded by broken lines in map *E* (1, 42). Presumably both the VSCT inhibition and the ascending discharge are mediated by the same functional group of pyramidal tract fibres (see Discussion). The area eliciting the ascending discharge included a lateral part of the anterior sygmoid gyrus. This suggests that this area is related to the motor area rather than the sensory area. It would follow that the inhibitory area, from which maximal effects were obtained close to the cruciate sulcus, also is related to the motor area.

It will be shown below (section 5) that pyramidal inhibition to VSCT is mediated by interneurones that also carry the inhibition from flexor reflex afferents to this tract. Presumably the discharge in VFRT is elicited through the interneurones that mediate excitation from the flexor reflex afferents to these neurones (see Discussion). The inhibition to VSCT neurones is supplied mainly by flexor reflex afferents in ipsilateral nerves (contralateral nerves provide only weak inhibitory and excitatory effects), whereas the excitation to VFRT is supplied symmetrically by the flexor reflex afferents of both sides (34, 35). This may explain why the pyramidal effects to VSCT were unilateral and to VFRT bilateral.

No inhibition of the VSCT discharge was observed from somatic area II. The ascending discharge from this area was small; possibly any inhibitory effect to VSCT was too weak to be detected.

Facilitation of the VSCT discharge could be produced from three areas (vertical hatching, maps *A*, *C*). The *caudal area* consisted of the most rostral part of the lateral gyrus. In some cases weak facilitation could be evoked more laterally than shown in map *A*, sometimes even laterally of the lateral sulcus. Maximal facilitation from the *rostral area* was usually obtained from the anterior sygmoid gyrus, the whole area extending widely over this gyrus and also including a rostral strip on the posterior sygmoid gyrus. Part of this area was concealed by inhibitory effects and

was revealed after pyramidotomy (map *C*; Fig. 3). Sometimes the facilitatory area extended more laterally than shown, but there were always gaps between this area and the other two. It had a more medial and rostral extension than the area from which the ascending discharge could be evoked. Weak facilitation could usually be produced from a *third area* corresponding to somatic area II.

The facilitation was either of equal strength from the rostral and caudal area, or weaker, in one case even absent, from the caudal area. The facilitation could be produced from symmetrical areas on the two hemispheres (map *A*) but the effect was usually (cf., however, Fig. 4) much weaker from the areas ipsilateral to the VSCT neurones. The facilitation from all areas remained after pyramidotomy (map *C*; Fig. 3) and was carried by fibres in the dorsolateral funiculus ipsilateral to the VSCT neurones (Fig. 4, *A-L*).

The facilitation to VSCT can, at present, not with any certainty be correlated with other functions of the cortex. However, it is of interest that Tower (38) was able to evoke extrapyramidal motor effects from regions approximately corresponding to the facilitatory areas.

4. *Thresholds and latencies.* — The maps in Fig. 5 were obtained at a fixed interval between conditioning and testing which gave maximal facilitation and inhibition. As a rule both facilitation and inhibition could be demonstrated from the inhibitory area, as is illustrated in Fig. 6 *A*. This gives additional evidence for overlapping facilitatory and inhibitory areas, besides that presented in the previous section. Facilitation preceded the inhibition even at liminal stimulus strength (Fig. 9 *A*, open circles). Similar findings were made in other experiments; the electrical threshold for facilitation and inhibition was approximately the same. Furthermore, there was no consistent difference between the threshold for inhibition, for facilitation from either caudal or rostral area, or for evoking an ascending discharge. The facilitation and inhibition reached a maximum when the stimulus strength was increased to about twice the threshold for a barely perceptible effect, whereas the ascending discharge usually continued to grow at higher relative strengths.

The latency of the facilitation was assessed in a number of experiments. In Fig. 6 *B* a single stimulus applied to the rostral facilitatory area sufficed to produce a recordable facilitation (crosses). The latency measured to the beginning of the VSCT discharge was

about 6.5 msec. Most of the VSCT neurones activated from the hamstring and triceps nerves are situated in the L_4 and L_5 segments (20, 33). The time for conduction from the VSCT cells to the recording place at the L_2 level can be calculated to less than half a

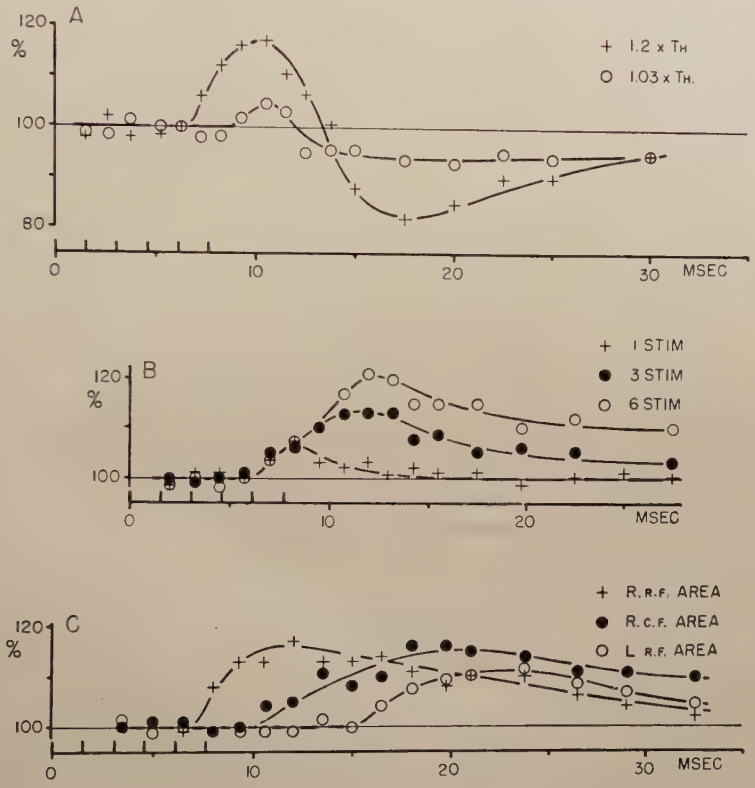


Fig. 6. – *Time courses of cortical facilitation and inhibition of VSCT mass discharge.*

A, facilitation and inhibition of the right VSCT discharge on repetitive stimulation (six stimuli) of the right "inhibitory" area. Two strengths of stimulation were used and are given as multiples of threshold strength producing a just perceptible effect on the VSCT discharge. *B*, facilitation of the right VSCT discharge on stimulation of the right rostral facilitatory area. The three curves were obtained with one, three, and siw stimuli as indicated. *C*, facilitation of the right *VSCT* discharge from three different areas: the right rostral facilitatory (RRF), the right caudal facilitatory (RCF), and the left rostral facilitatory (LRF) area. In all curves the abscissa represents the interval between the first conditioning stimulus and the beginning of the test discharge, and the ordinate, the height of the conditioned VSCT discharge in per cent of the unconditioned one. The stimuli are indicated above the line representing the abscissa. All the values were obtained from records consisting of about 10 superposed traces.

msec (cf. 9). Hence the facilitation of the VSCT neurones had a latency of slightly more than 6 msec. In other experiments the latency varied between 6.5 and 8 msec. An increased number of stimuli (Fig. 6 *B*) prolonged the facilitation but no paths with shorter latencies were opened up.

Longer latencies of facilitation were observed with other facilitatory areas. In Fig. 6 *C* the latency from the rostral area contralateral to the VSCT neurones was about 7.5 msec (crosses). A longer latency, about 10 msec, and later maximum characterized the facilitation from the contralateral caudal area (filled circles), and the latency from the ipsilateral rostral area was even longer, about 15 msec (open circles). These findings suggest pathways of different lengths from the different areas, possibly they either originate or are relayed in the rostral area contralateral to the VSCT neurones.

5. *Organization of inhibitory pathway at segmental level.* — It has previously been shown (36) that VSCT neurones are inhibited on repetitive stimulation of the dissected ipsilateral dorsolateral funiculus. This inhibition is carried by fibres of a high conduction velocity (up to 85 m/sec) and has a delay of several msec. The latter indicates that the inhibition is mediated by interneurones at the segmental level. Other fibre systems than the pyramidal tract may contribute to this inhibition but there is at present no need for such an assumption. We have now investigated further the organization of the interneuronal path from the pyramidal tract to the VSCT neurones.

VSCT neurones are strongly inhibited by volleys in ipsilateral flexor reflex afferents and by volleys in the ipsilateral pyramidal tract. These two polysynaptic pathways to the VSCT neurones were similarly influenced by the supraspinal control system described by Holmqvist *et al.* (18). This system consists of fibres originating from cells in the lower brain stem and descending in the dorsolateral funiculus; from each funiculus a bilateral action is exerted at the segmental level. It suppresses the transmission in a number of pathways activated from the flexor reflex afferents, including the inhibitory path to VSCT neurones. This control system is tonically active in the decerebrate preparation; in the spinal, or partially spinal preparation its action can be imitated by repetitive stimulation of the dissected dorsolateral funiculus (18, 36). In Fig. 7, the right VSCT discharge was uninfluenced by repetitive stimulation of the dissected right dorsolateral funiculus (*A*, *B*). Such stimulation

resulted in a marked decrease of the VSCT inhibition produced by cortical stimulation (*E*, *F*).

Stimulation of the dorsolateral funiculus in Fig. 7 was of moderate strength. With stronger stimulation it was often possible to suppress completely the inhibition from the flexor reflex afferents and from cortex to VSCT. However, both in the present type of preparation and in the spinal preparation (unpublished observations) such strong stimulation of the dorsolateral funiculus resulted in facilitation of the VSCT discharge, making it difficult to assess the amount of "disinhibition". This facilitation had a long latency (about 8 msec) and a slow time course (maximum after about 20 msec). It was presumably due to removal of tonic inhibition from segmental interneurones mediating effects from the flexor reflex afferents.

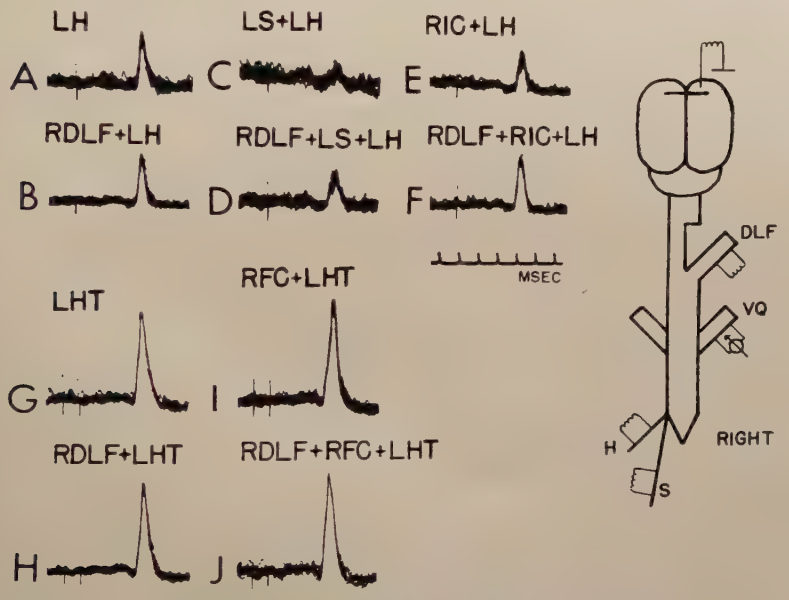


Fig. 7. – *Suprasegmental control of inhibitory pathway from skin afferents and from pyramidal tract.*

The stimulating and recording arrangement is shown by the diagram. The VSCT discharge was recorded from the right ventral quadrant (VQ) at L_2 and elicited by stimulation of the left hamstring nerve (LH, *A-F*) or by combined stimulation of the left hamstring and triceps nerves (LHT, *G-J*). The test VSCT discharge (*A*) was unchanged when preceded by repetitive stimulation (12 stimuli at 660 per second) applied to the right dorsolateral funiculus (RDLF) (*B*). *C* and *E* show VSCT discharges inhibited by stimulation of the left sural nerve (LS) and by repetitive stimulation of the right inhibitory cortex (RIC) respectively. Corresponding lower records (*D*, *F*) show that additional stimulation of RDLF results in a partial or complete disinhibition. Record *G* shows the test VSCT discharge which in I is facilitated by repetitive stimulation of the right rostral facilitatory area (RFC). Corresponding lower records (*H*, *J*) show that additional stimulation of RDLF (the same stimulating parameters as in *B*, *D* and *F*) did not alter the facilitation.

The possibility that the inhibition from the pyramidal tract and the flexor reflex afferents was mediated by common interneurones was investigated in experiments as that illustrated in Fig. 8. The graph shows the time course of cutaneous inhibition when tested on the ordinary VSCT response (crosses) and when tested on the VSCT response slightly inhibited by preceding cortical stimulation (open circles). In the latter case the initial part of the cutaneous inhibition was strongly facilitated by the cortical stimulation. It is concluded that the pyramidal tract and the flexor reflex afferents converge to common interneurones. At longer intervals the two curves in Fig. 8 coincide or deviate little from each others. Occlusion may be partly responsible though it was not favoured by the conditions in the experiment: both cortical and nerve stimulation were adjusted to give weak inhibition. Presumably inhibitory effects to the interneurones were partly responsible.

The results described in this section are summarized in the

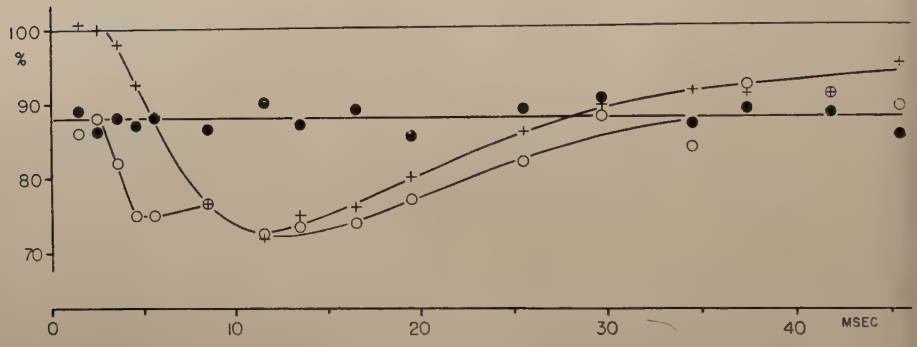


Fig. 8. – *Interaction of effects from cutaneous afferents and from inhibitory cortical area.*

The curves show the time course of cutaneous inhibition of VSCT neurones when tested on the ordinary VSCT response (crosses) and when tested on the VSCT response inhibited by cortical stimulation (open circles). Abscissa: interval between stimuli to sural and hamstring nerves. Ordinate: height of conditioned VSCT discharge in per cent of inconditioned one. At every tested interval four records consisting of about 10 superposed traces were obtained: 1) the control VSCT discharge elicited by combined stimulation of the hamstring and triceps nerves and used for calculating, in per cent, the effect of conditioning; 2) the VSCT discharge inhibited by cortical stimulation (10 stimuli, 660 per second) at a fixed interval (25 msec) before the discharge (indicated in per cent of control VSCT discharge, filled circles); 3) the VSCT discharge conditioned by stimulation of the sural nerve (crosses); 4) the VSCT discharge inhibited by cortical stimulation as in 2) and conditioned by stimulation of the sural nerve (open circles). Note that the black circles do not indicate a time course.

diagram shown in Fig. 9. The VSCT neurones are monosynaptically excited from I*b* afferents and polysynaptically inhibited from the flexor reflex afferents (the connection schematically indicated as a disynaptic path). The interneurones activated by the flexor reflex

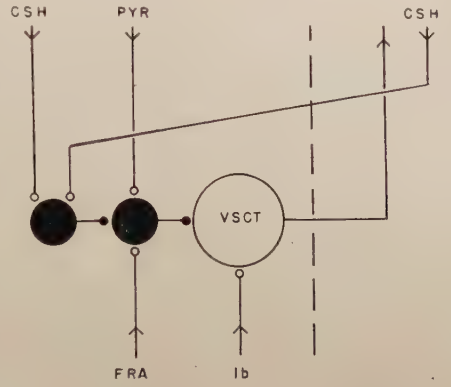


Fig. 9. – *Organization at segmental level of two descending control systems which act on the inhibitory pathway from the flexor reflex afferents to VSCT neurones.*

VSCT neurones indicated by large open circle. Excitatory synaptic terminals shown as open circles, inhibitory neurones and terminals, as filled circles. Polysynaptic connections are shown as disynaptic. Midline of spinal cord indicated by broken line. Abbreviations: Ib, Golgi tendon organ afferents; FRA, flexor reflex afferents; PYR., functional subdivision of pyramidal tract; CSH, supraspinal control system of Holmqvist *et al.* (18). See the text.

afferents are also activated by the ipsilateral pyramidal tract and inhibited by the ipsilateral and contralateral control system of Holmqvist *et al.* (18). This inhibition is presumably mediated by inhibitory interneurones as in other investigated pathways of the central nervous system (cf. 7).

6. *Organization of facilitatory pathway at segmental level.* — The cortical facilitation was carried by an extrapyramidal pathway. It suggests that this pathway is interrupted in the brain by at least one synapse. The short latency of the facilitation (about 6.5 msec, see section 4) indicates that it acts either monosynaptically, or through one, possibly two, interneurones, at the segmental level.

The cortical facilitation was never influenced by stimulation of the dissected dorsolateral funiculus (Fig. 7 *G-J*). It is concluded that segmental interneurones, if any exist, are not depressed by the control system of Holmqvist *et al.* One observation may suggest that interneurones are interpolated at the segmental level. The cortical facilitation was regularly abolished when the two inhibitory pathways to the VSCT were activated. In Fig. 10 *C*, stimulation of the sural nerve caused a large inhibition of the VSCT discharge

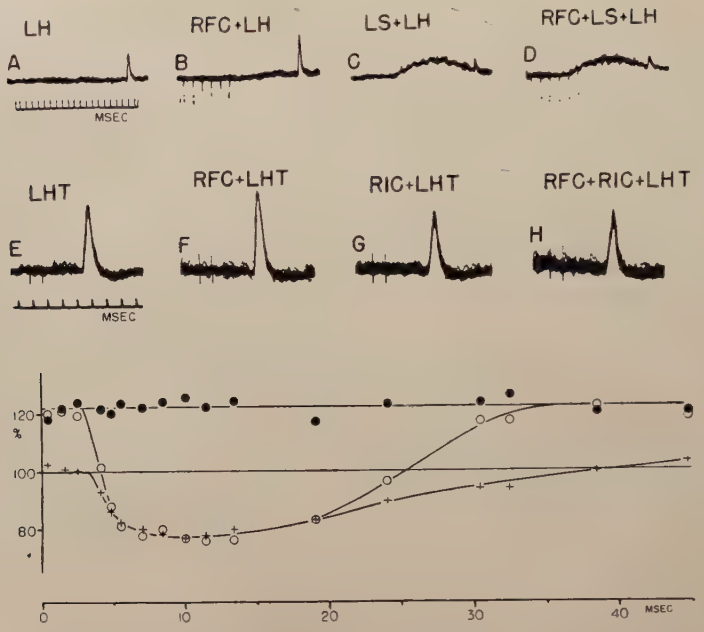


Fig. 10. – *Suppression of cortical facilitation concomitantly with inhibition of the VSCT.*

Record *A* shows the test VSCT discharge recorded from the right ventral quadrant on stimulation of the left hamstring nerve (LH). The VSCT discharge was conditioned, in *B*, by repetitive stimulation (all artefacts shown) of the rostral facilitatory area of the right cortex (RFC) and, in *C*, by a single stimulation of the left sural nerve (LS). The effect of combined stimulation of cortex and sural nerve is shown in *D*. *E-F* were obtained in a different experiment. The VSCT discharge was elicited by combined stimulation of the left hamstring and triceps nerves (LHT). The VSCT discharge is unconditioned in *E*, conditioned by repetitive stimulation (12 stimuli at 660 per second, preceding the sweep) of the right rostral facilitatory (RFC) and right inhibitory cortex (RIC) in *F* and *G* respectively. *H* was obtained on combined stimulation of the two cortical areas. The graph shows an experiment similar to that illustrated by records *A-D*. The time course of inhibition of the right VSCT produced by a single afferent volley in the left sural nerve was tested on the ordinary VSCT response (crosses) and on the VSCT response facilitated by repetitive stimulation of the right rostral facilitatory area (12 stimuli) at a fixed interval before the testing stimuli (open circles). Abscissa and ordinate, testing procedure, and significance of filled circles as in Fig. 8.

which is shown unconditioned in *A*. The inhibited VSCT response was not appreciably facilitated by cortical stimulation (*D*) that greatly facilitated the uninhibited VSCT discharge (*B*). A similar disappearance of the cortical facilitation was seen when the VSCT response was inhibited from the cortex (*E-H*). In *F*, the facilitation

was 25 per cent, whereas, in *G*, the cortical inhibition decreased the VSCT discharge by 20 per cent. Combined stimulation of facilitatory and inhibitory cortical areas (*H*) resulted in inhibition as large as that produced on isolated stimulation of the inhibitory area (*G*).

The graph in Fig. 10 shows the time course of the inhibition produced by a cutaneous afferent volley when tested on the ordinary VSCT response (crosses) and when tested on the cortically facilitated VSCT response (open circles). At intervals of 5 to 18 msec between conditioning and testing stimuli the curves coincide showing that the cortical facilitation was completely abolished. The decrease of the facilitation was apparent at an interval of 4 msec, when the

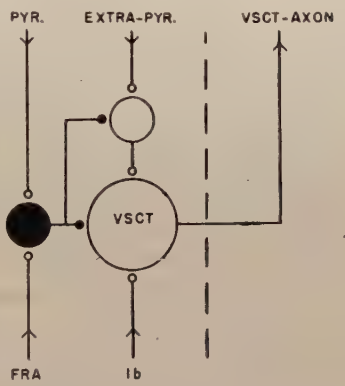


Fig. 11. – *Hypothetical organization at segmental level of the pyramidal and extrapyramidal pathways to VSCT.*

VSCT neurones indicated by large open circle. Excitatory interneurones and synaptic terminals shown as open circles, inhibitosy interneurones and synaptic terminals, as filled circles. Polysynaptic connections are shown as disynaptic (the pathway from the extrapyramidal system is probably disynaptic). Midline of spinal cord indicated by broken line. Abbreviations: Ib, Golgi tendon organ afferents; FRA, flexor reflex afferents; PYR., functional subdivision of pyramidal tract; EXTRA-PYR., extrapiramidal pathway. See the text.

facilitation was less than 10 per cent; *i.e.* the decrease had approximately the same latency as the inhibition of the VSCT neurones. Also in the other experiments there was close similarity in the time course of the VSCT inhibition and in the time course of the decrease of the facilitation. Some decrease of facilitation was usually detectable when the inhibition, either from the flexor reflex afferents or cortex, amounted to more than 10 per cent; there was usually complete suppression of the facilitation when it was more than 15 to 20 per cent.

The short latency of the decrease of the facilitation shows that it was due to interaction with the facilitatory pathway at the spinal level, presumably close to the VSCT neurones. The decrease occurred always concomitantly and in parallel with the inhibition produced in the VSCT neurones either by volleys in the pyramidal tract or in the flexor reflex afferents. It is concluded that these two systems are also responsible for the decrease of the facilitation. The diagram in Fig. 11 shows the most likely organization that would account

for the present results. The facilitatory pathway is disynaptically connected to the VSCT neurones. The interpolated neurones receive collaterals from the inhibitory interneurones that constitute the final common path for the inhibition from the pyramidal tract and the flexor reflex afferents. One has to assume further that the inhibitory action exerted on the postulated interneurones is much more potent than the inhibitory action exerted on the VSCT neurones. The only alternative to the mechanism postulated in the diagram would be presynaptic inhibition exerted on the facilitatory pathway (which then might be monosynaptic) (cf. 7, 8, 11, 12). This possibility seems less likely; it would be necessary to postulate an additional set of interneurones that were activated from both the pyramidal tract and the flexor reflex afferents.

Synaptic potentials in VSCT neurones were recently studied by intracellular recording technique in spinal preparations (9). Only inhibitory potentials were observed on electrical stimulation of the dissected dorsolateral funiculus ipsilateral to the tract neurones. These potentials can now be explained as due to stimulation of pyramidal tract fibres. The reason for the lack of depolarizing potentials, which would be expected from the present demonstration of a facilitatory pathway in the same funiculus, is not immediately obvious. The simultaneous stimulation of pyramidal tract fibres might result in suppression of the facilitation through the mechanism described above, or this mechanism might be tonically active in the spinal state. The activation of pyramidal tract fibres would presumably very effectively suppress any delayed part of the facilitation but would hardly occur early enough to suppress the initial part. The second explanation, a tonic suppression of the facilitation, is supported by the finding that repetitive stimulation of the control system of Holmqvist *et al.* (cf. preceding section) produces, in the spinal state, an increase of the VSCT response of up to 20 per cent (unpublished results). This increase is presumably due to inhibition of tonic activity in the interneurones that are interpolated on the path from the flexor reflex afferents and the pyramidal tract to VSCT neurones. Hence there is indication of a considerable tonic inhibition of the VSCT neurones in the spinal state. A corresponding inhibition induced by stimulation of the two inhibitory systems to VSCT in the intact preparation was usually accompanied by a complete suppression of the facilitation.

Discussion

1. *The inhibitory pathway.* — It has now been shown that volleys in the pyramidal tract inhibit ipsilateral (relative to the cell bodies) VSCT neurones. This explains why VSCT neurones are inhibited on repetitive stimulation of the dissected ipsilateral dorsolateral funiculus (36). The pyramidal inhibition of VSCT neurones could be explained as due to activation of interneurones on the inhibitory pathway from the flexor reflex afferents to the VSCT neurones (section 5). This finding should be considered in connection

with two other observation: the recent disclosure by Lundberg and Voorhoeve (29) that volleys in the pyramidal tract activate interneurones on the excitatory and inhibitory paths from the flexor reflex afferents to motoneurones, and the present finding that such volleys elicit a discharge bilaterally in the VFRT (section 1). It is most likely that this discharge is due to pyramidal activation of interneurones on the pathways that mediate excitation from the flexor reflex afferents to the ipsilateral and controlateral VFRT.

The similarity in the functional organization of the several pathways that are activated from the flexor reflex afferents has been emphasized repeatedly (15, 17, 18, 28). These pathways mediate excitation to ipsilateral flexor motoneurones and inhibition to ipsilateral extensor motoneurones (10), excitation to several ascending tracts, including VFRT, and inhibition to VSCT (18, 34, 35). The interneurones of these pathways are inhibited by the supraspinal control system described by Holmqvist and Lundberg (16) and by Holmqvist *et al.* (18). The present findings and those of Lundberg and Voorhoeve (29) suggest that these interneurones are excited by volleys in the pyramidal tract. It would follow that the pyramidal inhibition of the VSCT is the result of activity in a general control system with excitatory action on pathways activated from the flexor reflex afferents.

The functional subdivision of the pyramidal tract that influences the VFRT, and presumably also the VSCT, originates from the motor cortex rather than the sensory cortex (section 3). There is anatomical evidence suggesting that some ascending tracts are controlled by pyramidal tract fibres originating in the sensory cortex (22); the present findings suggest that some ascending tracts, presumably those concerned with information directly utilized in the regulation of motor functions, are controlled by the motor cortex.

2. *The facilitatory pathway.* — Facilitation of the VSCT discharge could be produced from three separate cortical areas (Fig. 5, maps *A*, *C*). The short latency of about 6.5 msec for the facilitation evoked from the rostral area contralateral to the VSCT neurones showed that this area had a relatively direct connection to the VSCT cells. The facilitation remained after pyramidotomy indicating that the pathway was extrapyramidal. Lesions in the spinal cord showed that the responsible fibres descended in the dorsolateral funiculus and exclusively exerted their action on ipsilaterel VSCT neurones. Further studies are needed to disclose the identity of the facilitatory system. One possibility to consider is the rubrospinal

tract which descends in the appropriate area of the cord, contains large fibres, and reaches the lower lumbar segments (2, 14, 31, 37). Corticorubral fibres arising from an area approximately corresponding to the rostral facilitatory area have been described (30).

The facilitatory system influenced VSCT but has no effect on the other investigated pathway, the VFRT. In contrast to the inhibitory system (see above) it may be specifically related to VSCT, or it may in addition influence uninvestigated pathways.

3. *Mechanisms for selection of information channels to VSCT.* — The VSCT is unique among investigated spinal tracts in that it receives information from two ascending systems with mutually antagonistic effects: Golgi tendon organ (I*b*) afferents with monosynaptic excitatory action, and flexor reflex afferents with polysynaptic, predominantly inhibitory, action. Ib afferents from nerves to many different muscles converge to ipsilateral VSCT neurones according to certain distinct patterns. These patterns indicate that the tract carries information concerning movements or positions at which these muscles contract together (9, 36). The flexor reflex afferents arise from very large receptive fields which include both hindlimbs and the caudal part of the abdomen (34). The centre of the receptive field consists of an ipsilateral cutaneous area together with underlying muscles; it supplies strong inhibitory action. The periphery of the receptive field, usually including part of the ipsilateral hindlimb, the contralateral hindlimb and part of the abdomen, supplies excitatory effects. These connections with the flexor reflex afferents presumably permits the VSCT to forward information concerning flexor reflex patterns (18, 28), the message being coded as inhibition and displayed against a background of resting activity (34). The ascending pathways to the VSCT neurones are diagramatically shown in Fig. 12. The connections from the flexor reflex afferents are schematically shown as disynaptic, and those from contralateral nerves which have weak linkages have been omitted. Inhibitory interneurones and their synaptic knobs are indicated by filled circles.

Four descending pathways which influence the VSCT neurones must now be recognized. They provide potential mechanisms for selection of one of the two information channels to the VSCT neurones, either the proprioceptive one or that informative of flexor reflex patterns. These descending systems and their postulated connections (see sections 5 and 6) are shown in Fig. 12:

i) The pyramidal tract (see section 5) excites the same interneurones that mediate inhibitory actions from the flexor reflex afferents. Presumably, on analogy with connections made to other pathways activated from these afferents (see Discussion, 1), also the interneurones on the excitatory pathway from the flexor reflex afferents receive similar excitation (not shown in diagram), though that remains unproved.

ii) The extrapyramidal pathway (possibly cortico-rubrospinal) is shown with a disynaptic linkage as postulated above (section 6). The interpolated interneurones receive collaterals from the inhibitory cells on the pathway from the flexor reflex afferents.

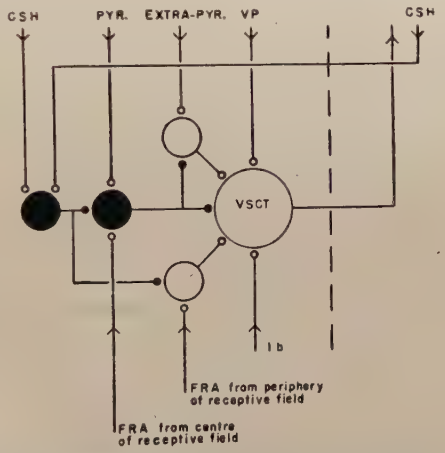


Fig. 12. – *Organization of three ascending and four descendign pathways to VSCT.*

VSCT neurones indicated by large open circle. Excitatory interneurones and synaptic terminals shown as open circles, inhibitory interneurones and synaptic terminals, as fidell circles. Polysynaptic connections are shown as disynaptic (the pathway from the extrapyramidal system is probably disynaptic). Midline of spinal cord indicated by broken line. Abbreviations: Ib, Golgi tendon organ afferents; FRA, flexor reflex afferents; PYR., functional subdivision of the pyramidal tract; EXTRA-PYR., extrapyramidal pathway; CSH, supraspinal control system of Holmqvist *et al.* (18); VP, monosynaptic pathway descending in the ventral quadrant. See the text.

iii) The supraspinal control system of Holmqvist *et al.* (18, cf. above, section 5) inhibits, via inhibitory interneurones, both the inhibitory (18, 36) and excitatory (36) path from the flexor reflex afferents. In contrast to pathways i), ii), and iv) this control system exerts its action bilaterally at the segmental level (18).

iv) A pathway located in the ventral quadrant and consisting of fast conducting fibres with monosynaptic excitatory connections to the VSCT neurones (9, 36). It has been suggested to originate from cells in the brain stem and to mediate effects from the cerebellum (19).

It has previously been demonstrated that, *in the spinal state* with interrupted descending pathways, the effects from the flexor reflex afferents dominate (34, 36). Isometric contraction of muscles

which very effectively activates tendon organs, invariably leads to inhibition of VSCT neurones. The flexor reflex afferents activated concomitantly with the tendon organ afferents supply inhibitory action which is stronger than the excitatory action supplied by the monosynaptic I*b* pathway. The inhibition is displayed against a background of resting activity which, in the spinal state, is supplied by the excitatory paths from the flexor reflex afferents. *In the decerebrate state*, the supraspinal control system (iii) of Holmqvist *et al.* is tonically active and very effectively suppresses the activity in the polysynaptic paths from the flexor reflex afferents (36). Isometric contraction of muscles now leads to excitation of the VSCT neurones. This excitation is, however, fairly weak and often insufficient to cause a discharge of the tract neurones. This may partly be due to a lowered excitability of the tract cells because of the suppressed transmission in the excitatory pathways from the flexor reflex afferents (cf. 36).

The interactions between the various pathways to the VSCT neurones have various consequences. When the control system (iii) of Holmqvist *et al.* is inactive, there are two mechanisms whereby the contrast between excitatory and inhibitory actions from the flexor reflex afferents can be increased. *a*) Activation of the inhibitory path from these afferents leads not only directly to inhibition of the VSCT neurones, but also indirectly through suppression of any facilitation mediated by the extrapyramidal tract (ii). *b*) Activity in the pyramidal tract (i) facilitates the inhibitory, and presumably also the facilitatory paths from the flexor reflex afferents. This would result in a higher resting activity against which a more powerful inhibition would be displayed. When the control system (iii) of Holmqvist *et al.* is active, the two pathways from the flexor reflex afferents are suppressed, whereas transmission in the extrapyramidal pathway (ii) is unrestrained (section 6). Facilitation from this system may well be necessary in order to permit the VSCT neurones to discharge when activated from the tendon organ afferents with their relatively weak synaptic linkage (34, 36).

There is no evidence that excitation from the descending monosynaptic pathway (iv) in the ventral quadrant is restricted by activity in other pathways. This system might serve as an independent regulator of the excitability of the VSCT neurones.

The interconnections between the control pathways constitute mechanisms whereby one system may gain complete dominance by

suppressing the others. This may be especially important when there are many control systems which influence the same tract. In the present case inhibition from the pyramidal tract may completely suppress the facilitation from the extrapyramidal pathway, whereas the control system of Holmqvist *et al.* may suppress all the effects exerted by the pyramidal tract.

Summary

The experiments were performed on cats under light pentobarbitone anaesthesia. The control from the cerebral cortex exerted on the transmission from primary afferents to the ventral spinocerebellar tract (VSCT) was investigated by mass discharge recording from the whole tract, and by microelectrode recording from VSCT fibres. Observations were also made on an ascending tract (VFRT) which is bilaterally activated from the flexor reflex afferents.

1) The pyramidal tract originating from the medial part of the posterior sygmoid gyrus (the hindlimb area of the sensorimotor cortex) inhibited VSCT neurones in the lumbar segments through segmental interneurones. These interneurones were, at least partly, identical with the interneurones that carry inhibition from the flexor reflex afferents to the VSCT cells. The inhibition was suppressed by activation of the supraspinal control system described by Holmqvist *et al.* (18).

2) Activity in the pyramidal tract evoked a discharge in the VFRT; the responsible cortical area included the hindlimb area of the sensorimotor cortex and a lateral part of the anterior sygmoid gyrus.

3) It is suggested that the two investigated pyramidal effects, inhibition to VSCT and excitation to VFRT, are mediated by a functional subdivision of the pyramidal tract which has an excitatory action on the various pathways activated from the flexor reflex afferents. It is further suggested that the motor cortex rather than the sensory cortex is the area of origin for these pyramidal fibres.

4) Facilitation of the VSCT was produced from three separate cortical areas (Fig. 8, map *C*), and carried by an extrapyramidal pathway descending in the dorsolateral funiculus ipsilateral to the cell bodies of the VSCT neurones. One of these facilitatory areas consisted of a large part of the anterior sygmoid gyrus, and the

rostromedial part of the posterior sygmoid gyrus. The facilitation from this area had a latency of about 6.5 msec, indicating a relatively direct pathway to the VSCT neurones.

5) An extremely powerful mechanism working at the segmental level could suppresse the cortical facilitation completely. This suppression appeared concomitantly with even slight inhibition of the VSCT when produced by either volleys in the flexor reflex afferents or the pyramidal tract. It is suggested that the interneurones which mediate the inhibition to VSCT are also responsible for the suppression of the facilitation.

6) The importance of the various supraspinal control systems, and their interaction, for the function of the VSCT is considered in relation to the two types of information which this tract has been postulated to carry.

REFERENCES

1. Adrian, A. D. Afferent discharges to the cerebral cortex from peripheral sense organs. *J. Physiol., 100*: 159-191, 1941.
2. Beusekom, G. T. van. *Fibre analysis of the anterior and lateral funiculi of the cord in the cat.* (Thesis.) Leiden, Eduard Idjo N. V., 1955.
3. Brodmann, K. Beiträge zur histologischen Lokalization der Grosshirnrinde. IV Mitteilung: Der Riesenpyramidentypus und sein Verhalten zu den Furchen bei der Karnivoren. *J. Psychol. Neurol., Lpz., 6*: 108-120, 1905.
4. Campbell, A. W. *Histological studies on the localization of cerebral function.* Cambridge, Cambridge University Press, 1905.
5. Chambers, W. W. and Liu C. N. Cortico-spinal tract of the cat. An attempt to correlate the pattern of degeneration with deficits in reflex activity following neocortical lesions. *J. comp. Neurol., 108*: 23-56, 1957.
6. Chiarugi, E., Rossi, G. F. and Zanchetti, A. The spinal course of the cortifugal fibres arising in the motor cortex of the cat. *Confin. neurol., 15*: 304-310, 1955.
7. Eccles, J. C. The nature of central inhibition. *Proc. Roy. Soc.* B, *153*: 445-476, 1961.
8. Eccles, J. C., Eccles, R. M. and Magni, F. Presynaptic inhibition in the spinal cord. *J. Physiol., 154*: 28 P, 1960.
9. Eccles, J. C., Hubbard, J. I. and Oscarsson, O. Intracellular recording from cells of the ventral spino-cerebellar tract. *J. Physiol., in press.*
10. Eccles, R. M. and Lundberg, A. Synaptic actions in motoneurones by afferents which may evoke the flexion reflex. *Arch. ital.Biol., 97*: 199-221, 1959.
11. Frank, K. Basic mechanisms of synaptic transmission in the central nervous system. *I.R.E. Trans. Med. Electron., ME-6*: 85-88, 1959.
12. Frank, K. and Fuortes, M. G. F. Presynaptic and postsynaptic inhibition of monosynaptic reflexes. *Fed. Proc., 16*: 39-40, 1957.
13. Garol, H. W. The " motor " cortex of the cat. *J. Neuropath., 1*: 139-145, 1942.
14. Hinman, A. and Carpenter, M. B. Efferent fibre projections of the red nucleus in the cat. *J. comp. Neurol., 113*: 61-80, 1959.
15. Holmqvist, B. Crossed spinal reflex actions evoked by volleys in somatic afferents. *Acta physiol. scand., 52* (suppl. 181): 1-67, 1961.

16. Holmqvist, B. and Lundberg, A. On the organization of the supraspinal inhibitory control of interneurones of various spinal reflex arcs. *Arch. ital. Biol.*, *97*: 340-356, 1959.
17. Holmqvist, B. and Lundberg, A. Differential supraspinal control of synaptic actions evoked by volleys in the flexion reflex afferents in alpha motoneurones. *Acta physiol. scand.*, in press.
18. Holmqvist, B., Lundberg, A. and Oscarsson, O. Supraspinal inhibitory control of transmission to three ascending spinal pathways influenced by the flexion reflex afferents. *Arch. ital. Biol.*, *98*: 60-80, 1960.
19. Holmqvist, B., Lundberg, A. and Oscarsson, O. A supraspinal control system monosynaptically connected with an ascending spinal pathway. *Arch. ital. Biol.*, *98*: 402-422, 1960.
20. Hubbard, J. I. and Oscarsson, O. Localization of the cells of origin of the ventral spino-cerebellar tract. *Nature*, *189*: 157-158, 1961.
21. Jabbur, S. J. and Towe, A. L. Analysis of the antidromic cortical response following stimulation of the medullary pyramids. *J. Physiol.*, *155*: 148-160, 1961.
22. Kuypers, H. G. J. M. Central cortical projections to motor and somatosensory cell groups. *Brain*, *83*: 161-184, 1960.
23. Lance, J. W. and Manning, R. L. Origin of the pyramidal tract in cat. *J. Physiol.*, *124*: 385-399, 1954.
24. Landau, W. M. An analysis of the cortical response to antidromic pyramidal tract stimulation in the cat. *EEG clin. Neurophysiol.*, *8*: 445-456, 1956.
25. Langworthy, O. R. The area frontalis of the cerebral cortex of the cat, its minute structure and physiological evidence of its control of the postural reflex. *Johns Hopk. Hosp., Bull.*, *42*: 20-60, 1928.
26. Laporte, Y., Lundberg, A. and Oscarsson, O. Functional organization of the dorsal spino-cerebellar tract in the cat. II. Single fibre recording in Flechsig's fasciculus on electrical stimulation of various peripheral nerves. *Acta physiol. scand.*, *36*: 188-203, 1956.
27. Livingston, A. and Phillips, C. G. Maps and thresholds for the sensorimotor cortex of the cat. *Quart. J. exp. Physiol.*, *42*: 190-205, 1957.
28. Lundberg, A. Integrative significance of patterns of connections made by muscle afferents in the spinal cord. *Proc. XXI int. physiol. Congr., Buenos Aires*, pp. 100-105, 1959.
29. Lundberg, A. and Voorhoeve, P. E. Pyramidal activation of interneurones of various spinal reflex arcs in the cat. *Experientia*, *17*: 46-47, 1961.
30. Mettler, F. A. The nonpyramidal motor projections from the frontal cerebral cortex. *Res. Publ. Ass. nerv. ment. Dis.*, *27*: 162-199, 1948.
31. Mussen, A. T. Experimental investigations on the cerebellum. *Brain*, *50*: 313-349, 1927.
32. Oscarsson, O. Functional organization of the ventral spino-cerebellar tract in the cat. I. Electrophysiological identification of the tract. *Acta physiol. scand.*, *38*: 145-165, 1956.
33. Oscarsson, O. Primary afferent collaterals and spinal relays of the dorsal and ventral spino-cerebellar tracts. *Acta. physiol. scand.*, *40*: 222-231, 1957.
34. Oscarsson, O. Functional organization of the ventral spino-cerebellar tract in the cat. II. Connections with muscle, joint, and skin afferents and effects of adequate stimulation of various receptors. *Acta physiol. scand.*, *42*: (suppl. 146): 1-107, 1957.
35. Oscarsson, O. Further observations on ascending spinal tracts activated from muscle, joint, and skin nerves. *Arch. ital. Biol.*, *96*: 199-215, 1958.
36. Oscarsson, O. Functional organization of the ventral spino-cerebellar tract. III. Supraspinal control of VSCT units of I-type. *Acta physiol. scand.*, *49*: 171-183, 1960.
37. Szentagothai-Schimert, J. Die Endigungsweise der absteigenden Rückenmarksbahnen. *Z. Anat. Entw-gesch.*, *111*: 322-330, 1942.

38. Tower, S. S. Extrapyramidal action from the cat's cerebral cortex: motor and inhibitory. *Brain, 59*: 408-444, 1936.
39. Ward, J. W. and Clark, S. L. Specific responses elicitable from subdivisions of the motor cortex of the cerebrum of the cat. *J. comp. Neurol., 63*: 49-64, 1935.
40. Woolsey, C. N. Some observations on brain fissuration in relation to cortical localization of function. In S. S. Tower and J. P. Schadè (Eds.): *Structure and Function of the Cerebral Cortex*. Amsterdam, Elsevier Publ. Co., pp. 64-68, 1960.
41. Woolsey, C. N. and Chang, H.-T. Activation of the cerebral cortex by antidromic volleys in the pyramidal tract. *Res. Publ. Ass. nerv. ment. Dis., 27*: 146-161, 1948.
42. Woolsey, C. N. and Fairman, D. Contralateral, ipsilateral and bilateral representation of cutaneous receptors in somatic areas I and II of the cerebral cortex of pig, sheep, and other mammals. *Surgery, 19*: 684-702, 1946.

ENHANCEMENT OF CORTICAL RESPONSES TO SHOCKS DELIVERED TO LATERAL GENICULATE BODY. LOCALIZATION AND MECHANISM OF THE EFFECTS [1]

A. ARDUINI AND M. H. GOLDSTEIN JR. [2]

Istituto di Fisiologia della Università di Pisa, Centro di Neurofisiologia del C. N. R., Sezione di Pisa and Communication Biophysics Group of Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A. [3]

INTRODUCTION

Previous experiments (2) showed that the response from the visual cortex to single shock stimulation of the lateral geniculate body was larger for conditions of visual deafferentation than for a dark-adapted retina. This enhancement was quite similar to that obtained during illumination of the retinas. It was suggested that the mechanism underlying both phenomena was the suppression or reduction of a tonic outflow of impulses from the dark-adapted retinas. In this interpretation the "dark discharge" influences the visual pathways in a way that reduces the size of the shock-evoked responses.

Suppression of the dark discharge not only leads to the forementioned effects in the visual pathways, but also underlies more generalized changes in the EEG patterns (1, 3). Here we ask whether

[1] The research has been sponsored jointly by the Office of Scientific Research of the Air Research and Development Command, United States Air Force, through its European Office, under contract No. AF 61(052)-107 and by the Rockefeller Foundation.

[2] Science Faculty Fellow of the National Science Foundation, U.S.A., on leave of absence from the Electrical Engineering Department, Massachusetts Institute of Technology, Cambridge, Mass.

[3] The research project is in common to the two Institutes. Animal experimentation has been carried on in Pisa, while analysis of the data stored on magnetic tapes has been performed at the Massachusetts Institute of Technology. Preliminary notes were published in: *Boll. Soc. ital. Biol. Sper.*, *36*: 1530-1532, 1960; and in *Research Laboratory of Electronics Massachusetts Institute of Technology, Quarterly Report*, nº 60, 215-221, 1961.

the enhancement of shock-evoked responses is restricted to the visual pathways or is, as it is the case for the EEG changes, more generalized.

An indication that brain structures other than specific visual pathways are involved comes from experiments on cats with midline sagittal splitting of the optical chiasm. In these preparations some enhancement of responses in the contralateral hemisphere was found with monocular illumination (2). Chang (7) has reported an enhancement of responses in the auditory cortex to shocks in the corresponding medial geniculate body during retinal illumination, suggesting a generalized phenomenon.

Another object of these investigations is to obtain further understanding of the mechanism of enhancement of responses during illumination and visual deafferentation. Accepting as a working hypothesis, still awaiting direct confirmation, that these effects have as a common origin the reduction or suppression of the dark discharge, we can ask whether the dark discharge produces a true Sherringtonian inhibition or whether there is a reduction in response due to occlusion. The second mechanism assumes an occlusion of neural elements at the geniculate and/or cortex arising from a tonic activity in these elements. The dark discharge would be the origin of this tonic activity and thus the origin of the occlusion.

For sake of simplicity, in the present paper we shall be concerned only with the earlier events following the single shock, the so-called " primary responses ". Later events, also time-locked to the stimulus, but generally included in the loose term " after effects " or " after discharges " will not be dealt with presently but will form the object of a further analysis.

To have a means of quantitative comparison of the effects obtained in different experimental conditions and to avoid errors due to the fluctuation of individual responses we have utilized the technique of averaging the evoked responses (see under: Methods).

Methods

Most of the experiments were made on cats with a complete transection of the brain stem at a midpontine level, just in front of the trigeminal roots. The technique of preparation has been previously reported (2). In some control experiments, cats under Nembutal anesthesia (intraperitoneal) were used, or Nembutal was injected intravenously in the midpontine preparation. Only in a minority of experiments where the animal's respiration was not satisfactory was artificial ventilation used. A few of the preparations were immobilized with Sincurarine.

Electrical shocks were applied to the lateral geniculate body, the optic tract, or the medial geniculate body, and activity recorded from the corresponding sensory projection areas. Electrical pulses, usually 0.2 msec duration and 2 V to 5 V amplitude, were delivered by a Tektronix stimulator through isolation transformers. The pulses were applied to the lateral geniculate body or the optic tract by bipolar, concentric electrodes, oriented stereotaxically, and usually to the medial geniculate body by bipolar electrodes about 1 mm apart, oriented anterior-posterior and at the same depth. The parallel bipolar arrangement was found to be more effective in stimulating the medial geniculate body than a concentric electrode. Histological control of the position of the electrodes was routinely performed with Nissl and Weil series stained preparations.

An acute and reversible deafferentation of the visual system was obtained by raising the intraocular pressure as described previously (1). Standard illumination was achieved by the beam of a 30 W filament lamp suspended 40 cm above the preparation, at about a 75° angle. The brightness of illumination was controlled by a diaphragm. In some experiments a flickering light was produced by chopping the light beam with a fan, the speed of which controlled the flicker rate. The fan blades were such that the "on" and "off" times of the flickering light were roughly equal.

Activity was recorded from the surface of the cortex through silver-silver chloride electrodes. In some, but not all, experiments the dura beneath the electrodes was removed. The potentials recorded from cortex and reticular formation were amplified by Grass Model P5 AC pre-amplifiers and recorded on magnetic tape by an Ampex Model FR-1107 recorder (7 channel, FM). The tape speed and preamplifier filters were set to give the system a pass-band from 1.5 c/s to 1250 c/s. A Grass Model IV EEG and Tektronix oscilloscopes were used to monitor activity during the experiments.

The potentials recorded on tape were processed at the Research Laboratory of Electronics, Massachusetts Institute of Technology, by means of a digital average response computer (ARC-1). The details of operation and application of this computer are given elsewhere (8, 9). Briefly, the computer sums the waveforms of activity following a number of stimuli, thus giving as its output the sum or (with appropriate scale factor) the average of the waveforms of the evoked responses processed.

The rate of stimulation was usually once every four seconds. In many experiments stimulation of medial geniculate body in one hemisphere was interleaved with stimulation of lateral geniculate body or optic tract of the opposite hemisphere so that the stimuli alternated with a time of two seconds between stimulation of one side and the other. The average of responses from the appropriate cortical areas can then be computed separately and compared with assurance that the general condition of the preparation is the same for both sets of responses. The same technique was used when anterior and posterior locations in a lateral geniculate body were stimulated in alternation, with the two sets of evoked responses averaged separately.

Results

1. *Effects of steady illumination and of deafferentation on the evoked responses from lateral and medial geniculate stimulation.* — In a set of experiments on the pretrigeminal preparation the lateral and the medial geniculate nuclei were stimulated with interleaved shocks 4/sec apart on each nucleus (see under Methods). The responses were led from the corresponding points of cortical areas

Visual I and Auditory I giving maximum amplitude of evoked potentials. The stimulation intensity was usually set slightly above the level where our monitor scope first showed a clear response with the characteristic waveform and thus was always well suprathreshold. The analysis of the average of a suitable number of responses showed

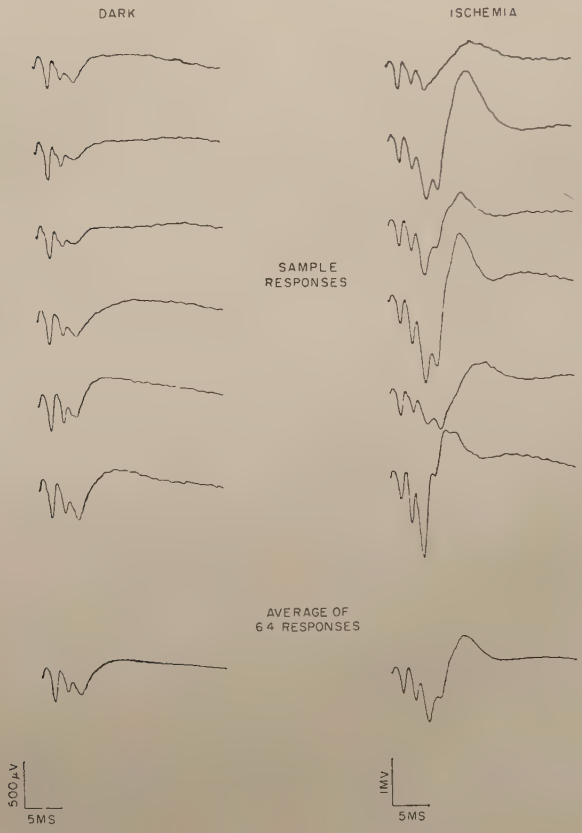


Fig. 1. – *Stability of the average of responses led from striate cortex after delivery of shocks to the corresponding lateral geniculate body.*

Start of each trace is synchronous with delivery of a shock. In the experiments illustrated shock amplitude was 5 V. The waveforms at the left were recorded during retinal conditions of dark adaptation; those on the right were recorded after application of intraocular pressure. Monopolar records; surface positive potentials are indicated by downwards deflections. (Midpontine pretrigeminal cat).

that those of the visual area were enhanced during illumination as well as during reversible retinal deafferentation. We have therefore confirmed the data reported in previous experiments (2).

Fig. 1 illustrates the waveform of the responses for the dark-adapted retina and during ischemia demonstrating the usefulness of the averaging technique in obtaining stable measures of the events in the response waveform. The growth of wave 1 following reversible retinal deafferentation ranged between factors of 1.5 and 3. In any preparation where the responses were enhanced by light and

by deafferentation, the enhancement by deafferentation was always greater.

We have been unable to confirm Chang's observation as far as the auditory area responses to medial geniculate stimulation are concerned. Under strictly controlled conditions, since we were comparing sets of responses obtained from the same cat over the same period (by the interleaving of the stimuli), only the visual

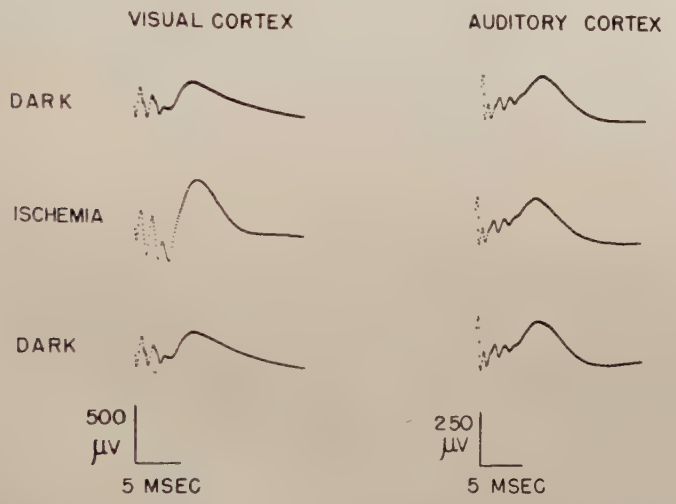


Fig. 2. – *Averages of cortical responses from shocks delivered to the lateral geniculate body and the medial geniculate body for different retinal conditions.*

Shock amplitudes were 5 V and 3 V, respectively. Averages of 64 responses for retinal conditions of dark adaptation, and of 32 responses for ischemia are shown. The set of responses averaged during ischemia was recorded approximately starting 30 sec after application of intraocular pressure. (Midpontine pretrigeminal cat).

area responses to lateral geniculate stimulation were enhanced. Fig. 2 illustrates the results when the enhancement of responses from the visual cortex was obtained by ischemia. The responses from the auditory area showed some degree of amplitude fluctuation but no significant changes in the averages of responses. There was also no average enhancement of auditory area responses during illumination which led to large enhancement of the visual area responses so that once again the phenomena with deafferentation and illumination were similar.

Hoping to resolve the discrepancy between our results and Chang's, the same procedure was followed in the barbiturate anesthetized preparation. However, even for moderately deep Nembutal anesthesia we were unable to observe any consistent change in size of the responses in the auditory area under steady illumination or visual deafferentation, although the enhancement of the responses led from the visual area was very marked in these experimental conditions.

2. *Characteristics of the averaged evoked responses in the visual areas.* — The waveform of the averaged response shows the well

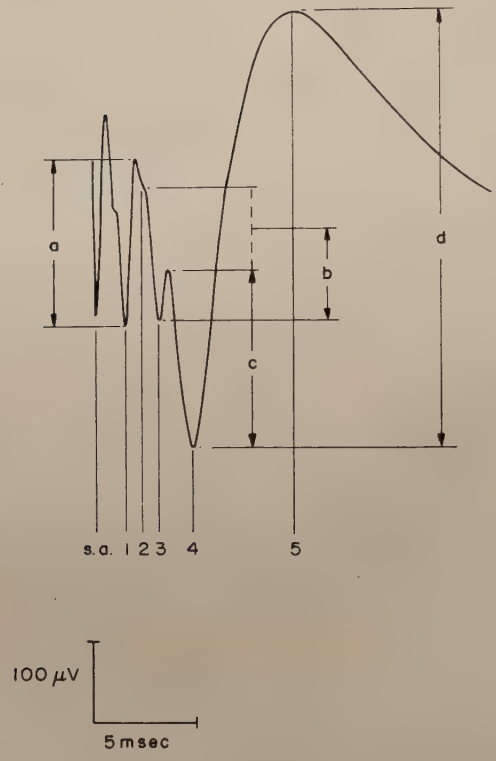


Fig. 3. – *Illustration of the waveform of the average of evoked responses.*

Average of 64 responses from Visual I cortex to 3 V shocks delivered to the lateral geniculate body. (Retinal conditions: dark adapted). The waveform shows the shock artifact (s. a.), and waves 1 to 5 in the evoked response numbered according to the usual scheme. Measures "a" to "d" represent an attempt to give a quantitative description of the principal features of the waveform. Measure "b" is between the positive peak of event 3 and a point midway between the inflection at the start of event 3 and the negative peak at the end of event 3. (Midpontine pretrigeminal cat).

known sequence of events. The events of the waveform are numbered according to the generally adopted convention (see 5, 6). In our records wave 2 was small or not detectable (see Fig. 3). The relative amplitude of the waves can differ considerably from one experiment to the other. The modification of the various events in the response waveforms during deafferentation and illumination

will be the topic of another report where particular attention will be given to a statistical analysis of the events.

Results, typical of the changes in the averaged waveforms are given in Table 1 which gives amplitudes of measures shown in Fig. 3 for various retinal and stimulus conditions. In this experiment the responses were enhanced both by deafferentation of the visual

TABLE 1. – *Growth of measures of the averages of evoked responses during ischemia and for an increase in shock amplitude.*

Measures on average of 64 responses in all cases except for figures in the second row for which 32 responses were averaged. See Fig. 3 for illustration of the measures and Fig. 6 for an illustration of the averaged responses in these conditions. (Midpontine pretrigeminal cat).

RETINAL CONDITION	SHOCK AMPLITUDE	COMPONENT SIZE in μV			
		a	*b*	*c*	*d*
Dark adapted	5 Volts	180	108	21	269
Ischemia	5 Volts	328	255	162	782
Dark adapted	5 Volts	164	104	18	257
Dark adapted	10 Volts	344	230	234	1170
Ischemia	10 Volts	521	416	625	2250
Dark adapted	10 Volts	364	242	281	1340

pathways and by increasing the intensity of the stimulus. Note that for both methods of enhancement the relative increase of measures *b* and *c* (corresponding to waves 3 and 4) is much larger than the increase in measure *a* (corresponding to wave 1).

3. *Dependence of enhancement upon location of stimulating electrode within the lateral geniculate nucleus.* — One might have speculated that the effect of enhancement of cortical responses would not be a generalized phenomenon, but it was a rather surprising finding that the effect was strongly dependent on the location of the stimulating electrode within the lateral geniculate nucleus. This was a chance observation in previous experiments which was more systematically investigated in the present ones.

The routine histological control of the electrode tracks showed that whenever the enhancement effects were strong the stimulating electrode was within the posterior three-fourths of the nucleus. The effects were almost absent or very weak when the anterior quarter was stimulated. After becoming aware of this, a posterior placement of the stimulating electrode was always sought.

As a control, a number of experiments were performed with two electrodes, one in the anterior and the other in the posterior part of the nucleus. The stimuli were delivered in alternation, once every 4/sec at each electrode, with two sec between alternate stimuli. Recording was from one location in the visual cortex. The average of evoked responses shows clearly that the potentials evoked by stimulating with the posterior electrode were enhanced to a considerable degree, whereas the responses evoked with the anterior stimulation were slightly, or not, affected (see Fig. 4). The distance

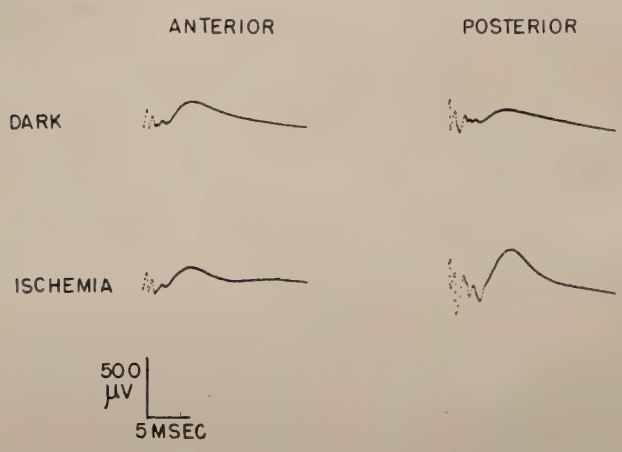


Fig. 4. – *Illustration of lack of enhancement of responses from shocks to the anterior lateral geniculate body while there is a sizable enhancement for shocks to the posterior lateral geniculate body.*

Each waveform is the average of 64 responses. Shock amplitudes were 3 V, anterior; 4 V, posterior. (Midpontine pretrigeminal preparation).

from tip to sleeve of the concentric electrodes, a little more than one-half a millimeter, did not permit us to discriminate whether the enhancement with posterior electrode placement was predominant in any of the three layers of the geniculate nucleus.

Stimulation of the optic tract prior to the lateral geniculate nucleus produced the enhancement effects under those same conditions in which the effects were obtained with stimulation of the posterior portion of the lateral geniculate body. It is worth emphasizing that the differential effects with stimulating electrode location were obtained both with illumination and with visual deafferentation.

4. *Enhancement of evoked responses in flickering light.* — This set of experiments was performed in order to further elucidate the mechanisms of the enhancement effects. The approach was to illuminate the eyes as was done to obtain the Chang effect, using, however, flickering instead of steady light (see under Methods).

The cortical responses evoked by electric shock stimulation of the lateral geniculate nucleus, obtained under conditions of different rates of flicker were then compared with those obtained in dark-adaptation. There were two constraints upon the physical stimulus. First, that the flicker frequency be the only variable parameter in order to avoid changes in response due to change in average intensity of illumination or to change in spectral content of the stimulus.

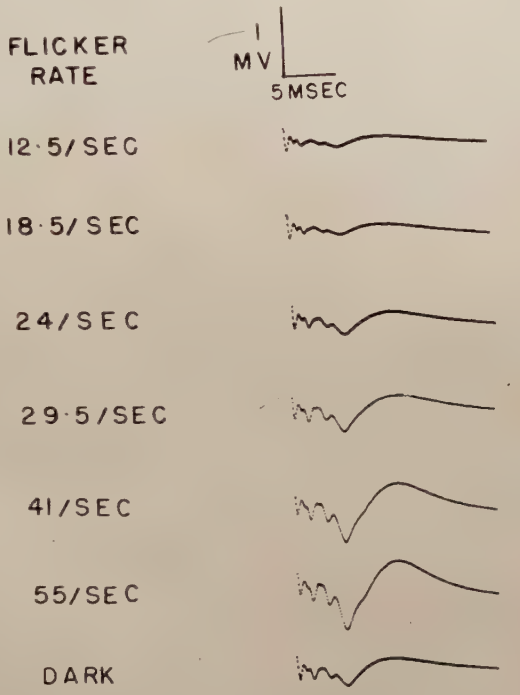


Fig. 5. – *Evoked responses to lateral geniculate body shocks during retinal illumination by a diffuse flickering light.*

Each record is an average of 64 responses. Control record during dark adaptation. Shock amplitude was 3 V. (Midpontine pretrigeminal cat).

This was accomplished by mechanically interrupting the beam by the blades of a variable speed fan. The second constraint was to use stimulus parameters that would avoid as much as possible any occlusion of the responses at cortical level. For the rates of flicker of 9/sec and higher, which were employed, the visual evoked response time-locked to the flickering light is small compared with the response to a weak or moderate shock to the lateral geniculate body. Yet, an occlusion at cortical level caused by a more or less asynchronous bombardment of impulses evoked by the flickering light stimulus is still possible (see under Discussion).

Results are illustrated in Fig. 5 and Table II, which gives the size of various measures of the averages of responses (see Fig. 3)

with the dark-adapted retina and with a number of flicker rates. It is evident that at a flicker rate of 12.5/sec all components of the averaged evoked responses are smaller than those obtained during dark-adaptation. A progressive enlargement of all components was

TABLE 2. – *Measures of waveforms of averaged shock-evoked responses obtained under retinal conditions of illumination by a flickering light and with dark adapted retinas.*

In each case 64 responses were averaged. See Figs. 3 and 5. (Midpontine pretrigeminal cat).

RETINAL CONDITION	COMPONENT SIZE in μv			
	a	*b*	*c*	*d*
Flicker 12.5/sec	90	31	58	235
Flicker 18.5/sec	110	45	72	250
Flicker 24/sec	175	98	190	478
Flicker 29.5/sec	270	170	320	730
Flicker 41/sec	300	240	540	1180
Flicker 55/sec	320	300	640	1410
Dark adapted	205	115	220	540

obtained by progressively increasing the flicker rate until a maximum enhancement, of about the same size as that produced by steady light, was obtained at flicker rates around 50-55/sec. For the flicker rate of 24/sec the average of the shock-evoked responses resembles closely in amplitude and waveform that obtained during dark-adaptation.

In a number of preparations which seemed in excellent physiological conditions and in which histological control indicated a completely successful section of the brain stem and a favorable electrode placement, we were unable, even with the highest rates of flicker (55/sec), to obtain an appreciable enlargement of the evoked responses above the control level obtained with the dark-adapted retina. In these same preparations we were also unable to induce any Chang effect with steady light. However, the low rate flicker (9/sec) reduced the size of the average of the evoked responses below the level obtained in the control (dark-adapted retinas) condition.

5. *Cases of failure to obtain enhancement of the shock evoked responses during illumination.* — As mentioned in the last paragraph, there were some preparations in which under conditions of illumination that we would expect to lead to enhancement, no enhancement occurred. This finding was not restricted to preparations in with

the flickering light was used. In experiments in which the shock evoked responses were obtained during illumination and during ischemia, it was sometimes found that little or no enhancement was produced when the illumination was attempted first. Ocular ischemia led to a great enhancement; after releasing the ischemia we waited until the responses returned to control (dark-adapted retinas) size and again recorded responses during illumination. This illumination after ischemia produced considerable enhancement of the responses. The possibility of enhancing the evoked responses with retinal illumination did not disappear in the further course of the experiment.

When the Chang effect is studied in anesthetized cats, level of anesthesia may affect the degree of enhancement obtainable. In one experiment we were unable to produce the Chang effect in a lightly anesthetized cat; however, following an increase in the anesthetic dose a strong enhancement was obtained.

6. *Some factors affecting the enhancement of shock-evoked responses.* — The degree of enhancement of evoked responses in the visual

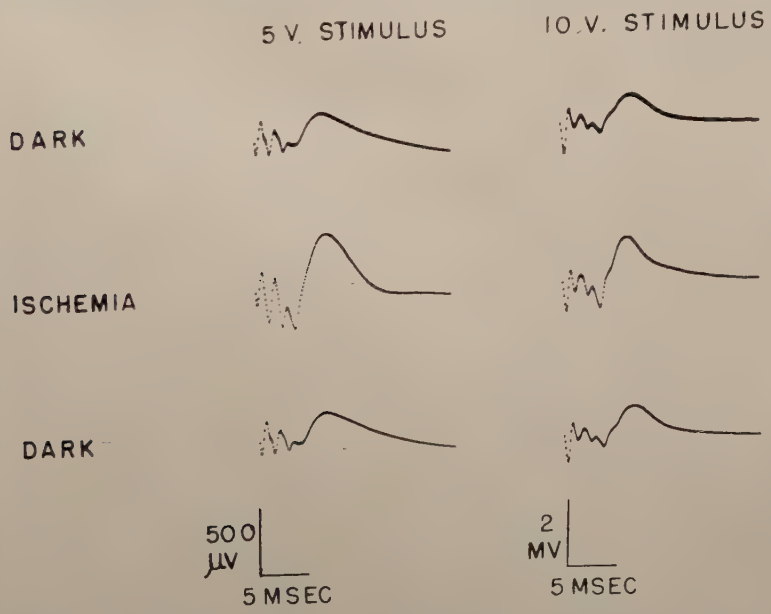


Fig. 6. – *Enhancement of cortical responses to shocks delivered to the lateral geniculate body for two shock amplitudes.*

Average of 64 responses for all records except for the middle left-hand column in which the average of 32 responses is shown. Note difference in amplitude calibration for the two columns. (Midpontine pretrigeminal cat).

area is influenced by a number of factors, including: i) location of stimulating electrodes within the lateral geniculate body (this result has been described in section 3); ii) retinal conditions, if we assume that the effects discussed in section 5 result from changes in the state of the retina; iii) the size of the stimulating pulses, since enhancement produced by illumination or ischemia was relatively greater when the responses were obtained with weak shocks producing near threshold control (dark-adapted retinas) responses (see Fig. 6).

Discussion

1. *Absence of enhancement of cortical responses to shocks to medial geniculate nucleus.* — We were unable to reproduce the enhancement of responses in the auditory area evoked by single shocks to the medial geniculate nucleus reported by Chang (7). In our experiments the average amplitude of responses from the auditory cortex remained constant while at the same time and in the same cat the cortical responses to single shocks to the lateral geniculate nucleus were strongly enhanced. This result was unexpected since, as has been described (2), the paths of facilitation are presumably not limited to the visual pathways. Moreover, since, under suitable conditions, widespread EEG changes follow retinal illumination or deafferentation (1, 3) the appearance of the enhancement effect outside the visual system would not have been surprising.

The averaging method we have employed excludes errors arising from measures of individual responses with widely varying amplitudes. We must conclude that Chang's observation of enhancement of the shock-evoked responses in the auditory pathways was related to factors more critical than those leading to the enhancement effects in the visual pathways.

2. *Differential effects depending on location of stimulating electrode within the lateral geniculate nucleus.* — The enhancement effects we observed were not only limited to the visual pathways but were also limited to responses elicited from the posterior three-fourths of the lateral geniculate nucleus. If this limitation held only for the enhancement effects following steady retinal illumination, the phenomenon could be ascribed to differential illumination of retinal areas. However, the influence of location of the stimulating electrode was most pronounced for enhancement during retinal deafferentation.

We must conclude that there is a particular arrangement of afferent fibers bringing the tonic dark discharge to the lateral geniculate nucleus.

The dimensions of our stimulating electrode did not permit localization within the three layers of the nucleus, other than the observation that the anterior placement was in that portion of the nucleus composed of the dorsal layer only (layer *A* in the anatomical terminology, see: 12). An accurate exploration of the three layers with electrodes having higher localizing power would be necessary and desirable to further elucidate the relationship of the physiological events to the anatomical structure.

3. *Experiments with flickering light.* — Two interpretations of the enhancement effect during retinal illumination have been advanced: 1) that the *activation* of retinal units brought about by steady illumination lowers the threshold of the postsynaptic neurons of the lateral geniculate body (7); and 2) that the enhancement of evoked responses is due to *reduction or suppression* of the afferent tonic activity (the dark discharge) originating in the retina (2). The second hypothesis was advanced when it was found that retinal deafferentation by ischemia produced enhancement effects similar to those obtained during retinal illumination. Our experiments with illumination by flickering rather than steady light serve to further test these two hypotheses.

At high rates of flickering (above about 50/sec) the retinal elements are quite insensitive to the modulation of the stimulus and results are completely the same as obtained under conditions of steady retinal illumination. At the lowest rates we have used (about 10/sec) it is reasonable to assume that some retinal elements discharge to each "on" of the light and some discharge to each "off" (see 10, 11). As the flicker rate is increased progressively the retinal activity will become less synchronized with the modulation of the stimulus, until finally there will only remain the asynchronous activity of those elements which respond to a steady light. Experimental evidence from microelectrode studies of the retina indicates a higher impulse traffic in the optic nerve and tract at our low flicker rates than at our high flicker rates. Thus the first hypothesis above predicts an increase of shock-evoked responses with decreasing flicker rate and the second hypothesis predicts the opposite. Our results are entirely compatible with the second hypothesis. As flicker rate is progressively decreased the shock-evoked

responses decrease progressively until at low rates the responses are smaller than those obtained with the dark adapted retina.

Thus, we conclude that the amplitude of cortical responses to shocks delivered to the lateral geniculate body is inversely related to the impulse traffic in the optic pathways. The hypothesis may be advanced that the enhancement of the cortical responses following i) steady illumination; ii) high rate repetitive photic stimulation and iii) retinal ischemia are related to a single cause, *viz.* reduction or abolition of the retinal dark discharge. The depressing effect of the dark discharge on the responses to geniculate shocks might by due either to true inhibition or to an occlusion effect. The experiments of Söderberg and Arden (14) showing an increase of the spontaneous single unit discharge of the lateral geniculate neurons, during visual deafferentation, would seem to support the inhibitory hypothesis.

4. *Difficulties in obtaining the enhancement effects during retinal illumination.* — When the enhancement effect is obtained in the same preparation with retinal deafferentation and with illumination, all other experimental variables being constant, the enhancement is always greater with deafferentation. This finding may be easily explained by the fact that some retinal elements are active during steady illumination, although the traffic seems to be decreased with respect to the dark adapted animals (13, 11). As stated in section 5, on some occasions, no enhancement could be obtained upon illumination, although the effect of retinal ischemia was clear-cut. If the intraocular pressure was then brought to normal levels after a short time, the cortical responses returned to control size; a strong enhancement effect was then obtained with the same illumination that failed to produce any upon first presentation. It is likely that the retinal population whose tonic discharge is decreased by steady illumination represents only a fraction of the population which is tonically active in complete darkness and which is inactivated by the ischemia. Following temporary retinal black-out, the fringe of units affected by steady light may be increased. Another differential effect was observed in a cat which gave no enhancement with retinal illumination when it was lightly anesthetized; such an effect, however, clearly appeared following a further dose of nembutal.

5. *Locus of the enhancement effect.* — Our findings reinforce the hypothesis that enhancement of the shock-evoked responses during retinal deafferentation and illumination results from a release from a depressant effect set up by the dark discharge. The

question of whether this depressant effect is operant only in the geniculate nucleus or whether it directly affects the cortex as well is most relevant.

The different components in the shock-evoked response have been related to discharges of the radiation fibers (wave 1) and to intracortical events (waves 3, 4 and 5) (cf. 4, 6, 15), so that a detailed study of the statistics of components of the evoked response waveform, along with more direct studies of neural activity in the visual pathways may lead to further understanding of the locus and mechanisms of the effects. Such studies are under way and will be reported separately.

Summary

Our experimental results may be summarized as follows:

1) Retinal conditions (deafferentation and illumination) which produce an enhancement of the responses led from the visual cortex following single shocks applied to the lateral genicule body, do not significantly increase the responses of the auditory cortex to a medial geniculate volley. Thus, these effects are less generalized than the changes of EEG patterns which follow retinal deafferentation in the midpontine pretrigeminal preparation.

2) Even within the visual system, no enhancement is observed when the stimulating electrode is in the anterior quarter of the lateral geniculate nucleus.

3) If flickering light is used, at high rates of flicker (above 50/sec) results are the same as with steady illumination. As the flicker rate is decreased the amplitude of the shock evoked responses decreases until, at low rates of flicker, the responses are smaller than those obtained with the retinas dark-adapted.

These findings support the hypothesis that enhancement effects obtained during steady retinal illumination or deafferentation result from a decrease in the neural traffic in the optic tracts. In control conditions (dark-adapted retinas) this neural traffic originates in the retinal dark discharge. A reduction or the complete temporary abolition of the retinal dark discharge may increase the shock evoked cortical response i) by releasing the geniculo-cortical system from a tonic inhibitory influence exerted by the dark discharge or ii) by reducing or abolishing the occlusion phenomena produced by the tonic activity.

REFERENCES

1. ARDUINI, A. and HIRAO, T. On the mechanism of the EEG sleep patterns elicited by acute visual deafferentation. *Arch. ital. Biol.*, *97*: 140-155, 1959.
2. ARDUINI, A. and HIRAO, T. Enhancement of evoked responses in the visual system during reversible retinal inactivation. *Arch. ital. Biol.*, *98*: 182-205, 1960.
3. ARDUINI, A. and HIRAO, T. EEG synchronization elicited by light. *Arch. ital. Biol.*, *98*: 275-292, 1960.
4. BISHOP, G. H. and CLARE, M. Radiation path from geniculate to optic cortex in cat. *J. Neurophysiol.*, *14*: 497-505, 1951.
5. BISHOP, G. H. and O'LEARY, J. Potential records from the optic cortex of the cat. *J. Neurophysiol.*, 1: 391-404, 1938.
6. BREMER, F. et STOUPEL, N. Analyse oscillographique comparée des réponses des aires de projection de l'écorce cérébrale du chat. *Arch. ital. Biol.*, *95*: 1-19, 1957.
7. CHANG, H. T. Cortical responses to stimulation of lateral geniculate body and the potentiation thereof by continuous illumination of the retina. *J. Neurophysiol.*, *15*: 5-26, 1952.
8. CLARK, W. A. Jr., BROWN, R. M., GOLDSTEIN, M. H. Jr., MOLNAR, C. E., O'BRIEN, D. F. and ZIEMAN, H. E. The average response computer: a digital device for computing averages and amplitude and time histograms of electrophysiological responses. Transact. PGME-IRE BME-8, 46-51, 1961.
9. Communication Biophysics Group of Research Laboratory of Electronics and W. M. SIEBERT: *Processing Neuroelectric Data.* Technical Report 351. Massachusetts Institute of Technology, Research Laboratory of Electronics, Cambridge, 1959, 121 pp.
10. GRANIT, R. *Sensory mechanisms of the retina.* London, Oxford University Press, 1947, 412 pp.
11. GRANIT, R. *Receptors and sensory perception.* New Haven, Yale University Press, 1955, XII-369 pp.
12. KAPPERS, C. U. A., HUBER, G. C. and CROSBY, E. C. *The comparative anatomy of the nervous system of vertebrates including man.* New York, MacMillan, 1936, II vols.
13. KUFFLER, S. W., FITZHUGH, R. and BARLOW, H. B. Maintained activity in the cat's retina in light and darkness. *J. gen. Physiol.*, *40*: 683-702, 1957.
14. SÖDERBERG, U. and ARDEN, G. B. Entlandungen einzelner Neurone im Geniculatum Laterale bei experimenteller Epilepsie. In R. JUNG ed. *The visual system: physiology and psycophysic.* Goettingen, Springer Verlag, in press.
15. WIDÉN, L. and AJMONE-MARSAN, C. Unitary analysis of the responses elicited in the visual cortex of cat. *Arch. ital. Biol.*, *98*: 248-274, 1960.

EFFECTS OF STROPHANTIN G AND AN α (β-PALMITOIL) - LYSOLECITHIN ON THE MECHANICAL ACTIVITY AND ON THE POTASSIUM TRANSFER IN THE ISOLATED, BEATING HEART OF RANA ESCULENTA

F. MARRO AND V. CAPRARO
Istituto di Fisiologia generale, Università di Parma, Italia

INTRODUCTION

It is known that digitalis glycosides modify the mechanical activity of the heart increasing its contractile energy. The mechanism of action of these drugs is not yet clear. They might increase the capacity of the contractile proteins to convert the chemical energy at their disposal into mechanical work (6). This phenomenon could take place as a result of an action on the proteins carried out directly or indirectly through a modification of the endocellular ionic ambient. In fact, it has been ascertained that the glycosides lead to a net loss of potassium (6). Furthermore, as Stutz and his colleagues showed, digitalis does not effect the tension of glyceric extracts of myocardium, in which there is no membrane activity, and this leads one to think that the action of these compounds depend, at least in part, on possible variations of such an activity (24).

The mechanism of action of these drugs on the heart then, may be referred to the problem of the relationship between mechanical activity, on the one hand, and ion content and transfer on the other. It is not yet clear whether, and how these phenomena are connected. A methodic analysis of the changes caused by the digitalis glucosides on this aspect of the cardiac activity would be very interesting.

It has been shown that in the blood and in the tissues there are substances which are able to effect the heart in a manner similar to the digitalis glycosides (19, 18, 3, 7). The active part of such substances seems to be identified with an α-(β-palmitoil)-lysolicithin (8). The similarity of the cardiac action of these substances and of strophantin has been reconfirmed by mean of an α-(β-palmitoil)-lysolecithin obtained from the egg lecithin through enzymatic hydrolysis by Russel snake venom and then purified (20). This substance has a strong haemolitic action and presumably a permeabilizing effect on the cells, as well as on the erythrocytes. It was interesting to study the above mentioned problem on the isolated, beating heart of rana esculenta, both with strophantin G and with the α-(β-palmitoil)-lysolecithin [1]. First, the effect of these two substances has been analysed on the mechanogram and on the rate of outflow of Rb^{86} in hearts previously equilibrated with Ringer solution in which part of the potassium had been substituted with this ion. Secondly, the effect of the two substances has been studied on mechanogram and on the potassium content of hearts perfused for different periods with normal Ringer and with Ringer containing the substances under examination.

Methods

The equipment we used in our experiments is shown in the diagram of Fig. 1.

The heart was prepared according to Straub's classic technique, using a cannula fitted with a lateral tube. A glass capillary tube, connected by a rubber junction to a reservois containing the perfusion fluid, was introduced into the cannula. A glass cylinder with an open bottom was adapted to the lower part of the cannula and provided with a lateral tube: this was connected to an oxygen cylinder through a humidifier so that the surface of the heart was placed in a saturated water vapour ambient. The cardiac contraction was recorded mechanically on an electrokymograph. The perfusion fluid was a Ringer solution containing NaCl 6.5 g ‰, KCl 0,15 g ‰, $CaCl_2$ 0,25 g ‰, $NaHCO_3$ 0.20 g ‰, glucose 1 g ‰.

Before the commencement of the experiment, the hearts were subjected to a preliminary perfusion for 60 min. Previous research had proved that, after mounting the preparation, the endocardiac potassium content initially decreased, reaching it lowest concentration after 20 min. Then the potassium level returned to values near to those of non-perfused hearts; this increase seems complete about 60 min after the commencement of the perfusion (Fig. 2) (16).

---

[1] Kindly supplied by the Istituto Carlo Erba per Ricerche Terapeutiche, Milano.

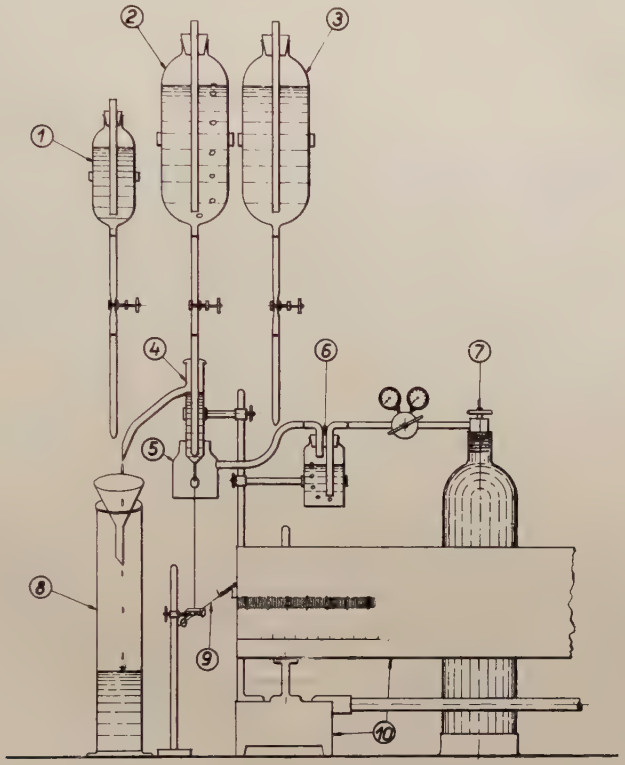


Fig. 1. - *Equipment used in the experiments.*

1, 2, 3: reservoirs for the solutions; 4: cannula; 5: glass cylinder; 6: humidifier; 7: O_2 cylinder; 8: cylinder for collecting the perfusion fluid; 9: stylus; 10: electrokymograph.

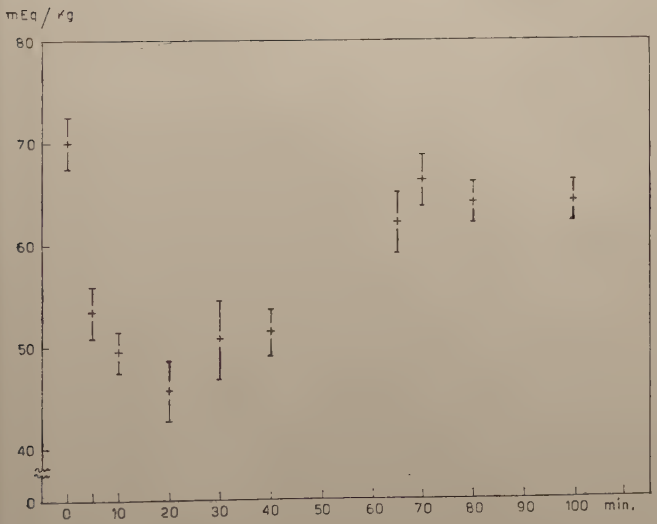


Fig. 2. – *Behaviour of the endocardiac in K^+ hearts perfused with normal Ringer as control.*

In the diagram are plotted the average values for groups of 5 hearts and their standard error. Ord.: endocardiac K^+, in mEq/Kg of fresh tissue. Abs: time in min.

Results

1. *Experiments on the modifications of the mechanical activity and of the kinetics of the Rb^{86} outflow.* — The experiments were carried out in the following way. After the preliminary equilibration, the hearts were perfused for a period of 40 min, with Ringer solution in which part of the potassium had been substituted with an isomolar amount of radioactive rubidium (Rb^{86} received from the Radiochemical Centre, Amersham, England, as RbCl). Previous experiments had in fact shown that the endocardiac radioactivity reached a steady value within a period of 30-40 min. At the end of this period the perfusion fluid was substituted with rubidium-free solution which was passed through the cannula at a constant speed of 8 ml/min. This speed was selected mainly in order to prevent the possible delay in washing out the marked ion released from the heart and its consequent accumulation in the cannula. Tests carried out in advance with increasing rate of flow had shown that, in relation to the capacity of our cannula, rates of flow above 2 or 3 ml/min did not increase any further the rate of the Rb^{86} outflow.

At fixed intervals, samples of perfusions were collected from the cannula into marked test tubes. The first samples, coincident with a period when the rate of the Rb^{86} outflow is very high, were taken every 15 sec, the subsequent ones, every 1 min. The volume of the samples was brought up to 10 ml and then the radioactivity of these was measured by means of a Geiger counter type M 6 XXth Century Electronics and Scaler DC 331 R made by Selo. The radioactivity measured for each sample corresponds to the amount of ion released from the heart into the solution throughout the collection of the sample and is expressed as a number of counts per minute.

The experiments have been carried out on three different groups, each of 5 hearts. In the first group the wash-out was done entirely with normal Ringer in order to test the kinetics of the Rb^{86} outflow in pharmacologically untreated hearts; in the second and third groups, after 30 min of wash-out, the normal Ringer solution was substituted with a Ringer solution containing strophantin-G 2 γ/ml and, α-(β-palmitoil)-lysolecithin 10 γ/ml repectively. In each case the whole wash-out was maintained as long as 70-90 min; the mechanical activity of the heart was recorded for 30 sec in coincidence with each collection of samples. The experiments were carried out

in the months of february and march with a room temperature of $20^0 \pm 2^0$ C.

The results [1] are illustrated in Figs. 3 and 4 which refer to the effects of strophantin-G and α-(β-palmitoil)-lysolecithin respectively,

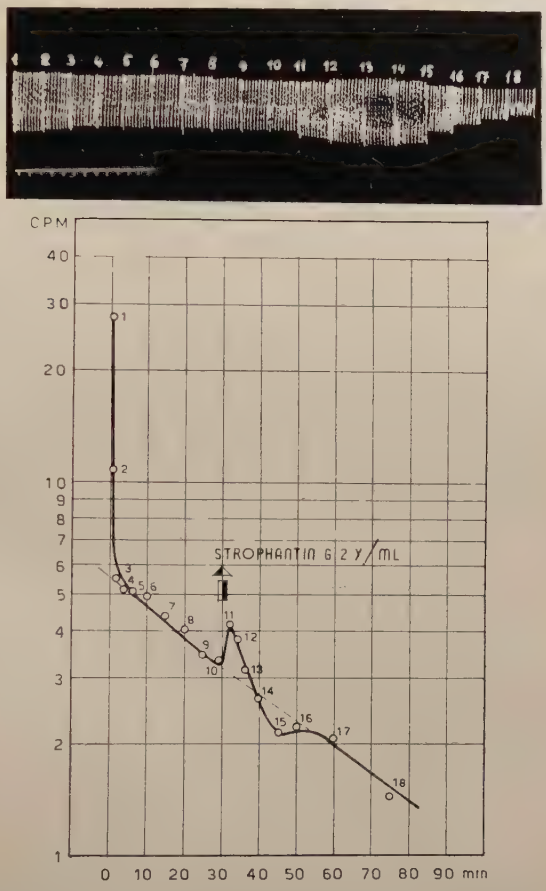


Fig. 3. – *Effects of strophantin G on the mechanical activity and on the Rb^{86} outflux.*

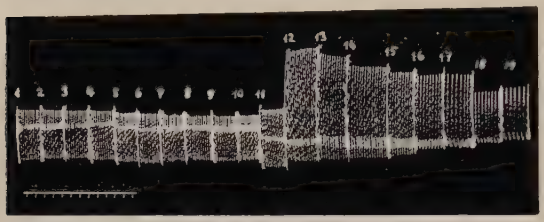
Top: cardiogram. Bottom: curve showing the kinetics of the Rb^{86} outflux. Ord.: radioactivity of the wash-out solution in counts per minute (cpm) 10^{-2}. Abs: time in min. The figures on the cardiogram and those on the points of the outflow curve show the time correspondence of recording mechanical activity and sampling the wash-out solution.

and show the mechanogram and the curve of the Rb^{86} outflow. On the ordinate, on logarithmic scale, the radioactivity of the wash-out is plotted in counts per minute 10^{-2}; on the abscissa the time in minutes. The broken line represents the kinetics of the Rb^{86} outflow from the untreated hearts. Equal figures on the mechanograms and on the single points of the outflow curve, respectively,

[1] Preliminary note in (2).

show the correspondence of time between the recording of the mechanical activity and collection of the wash-out sample.

From the diagrams, it appears that both drugs induce on the mechanical activity of the heart, a positive inotropic and tonotropic



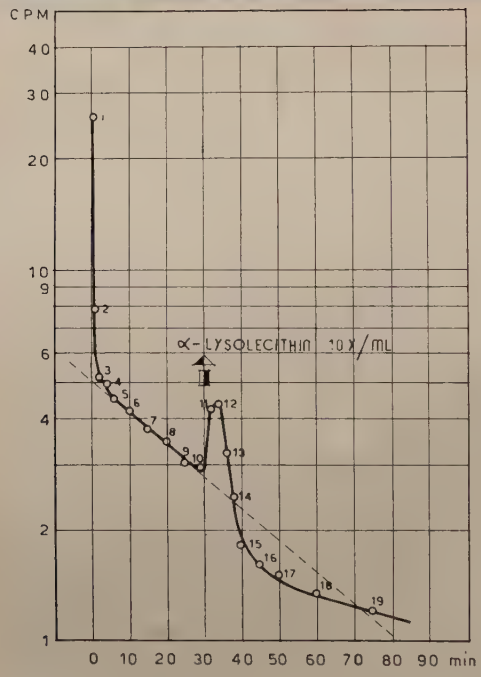


Fig. 4. – *Effects of α-(β - palmitoyl) - lysolecithin on the mechanical activity and on the Rb^{86} outflux.*

Top: Cardiogram. Bottom: curve showing the kinetics of the Rb^{86} outflux. Ord.: radioactivity of the wash-out solution in cpm 10^{-2}. Abs: time in min. The figures on the cardiogram and those on the points of the outflow curve show the time correspondence of recording mechanical activity and sampling the wash-out solution.

and a negative chronotropic effect which tends to become successively a contracture; simultaneously they cause a temporary increase in the rate of the outflow of Rb^{86} which occurs at the commencement of the cardiotonic effect and is stronger with the lysolecithin than with the strophantin G.

2. *Experiments on the modifications of the mechanical activity and on the endocardiac content of potassium.* — The experiments were carried out in the following way. After the preliminary equilibration, the perfusion was started with normal Ringer solution or with Ringer solution containing strophantin-G 2 γ/ml or α-(β-palmitoil)-lysolecithin 10 γ/ml. For each type of perfusion four groups, each of 4 hearts were used. These were subjected to periods of perfusion of 5, 10, 20 and 40 min respectively. In each experiment, the mechanogram was recorded for 30 sec during each minute of perfusion. At the end of the experiments, the hearts were weighed and then kept at 120° C for 12 hours. Thereafter they were treated with nitric acid and the potassium content of the resultant solution was measured by a Beckman DU sprectrophotometer. In addition, the intracardiac content of potassium was measured in excised, non-perfused hearts. All these experiments were carried out in the months of june and july at the temperature of 20° ± 2° C, which was kept constant by thermostating the perfusion fluid.

The results relative to the intracardiac content of potassium (described in a preliminary note, 14) are reported in the table and plotted in Fig. 5 as average values of the various groups of examined hearts. K+ content of the non perfused hearts: 68,79 ± 2,56. K+ content of the perfused hearts:

	5 min	10 min	20 min	40 min
Ringer . . .	61,77 ±3,06	65,8 ±2,44	64,19 ±2,23	63,57 ±2,02
Ringer + Strophantin-G.	63,15 ±1,81	63,23 ±6,48	63,93 ±6,91	48,17 ±3,1
Ringer + . . . Lysolecithin . .	62,20 ±2,61	63,65 ±1,47	63,18 ±6,81	51,30 ±1,38

Both in the table and in the graph, the potassium content is expressed in mEq/kg of fresh tissue and the variability is expressed as a standard error.

From the results, it first appears that in the controls perfused with normal Ringer solution, the content of potassium remains constant at least throughout the period of examination. It is lower than that of the nonperfused hearts with an average of 8%; and this value is within the limits of the net loss of potassium which one observes in isolated structures. Furthermore, the potassium content of the hearts perfused with Ringer solution containing the

drugs under examination and that of the hearts perfused with normal Ringer solution remains identical up to 20 min of perfusion. However, after 40 min of perfusion, the potassium content of the hearts perfused either with strophantin G or Lysolecithin Ringer solution shows a considerable and statistically significant decrease with respect to that of the hearts perfused with normal Ringer ($p < 0{,}01$);

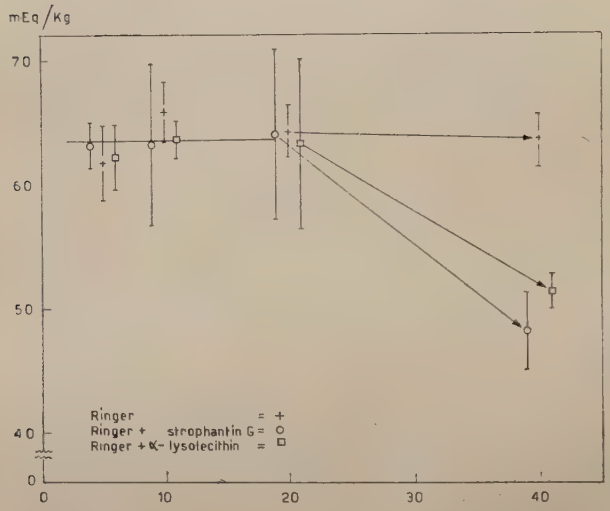


Fig. 5. – *Behaviour of endocardiac K^+ in hearts which were first perfused with normal Ringer for 60 min and then respectively with normal Ringer (controls), strophantin G Ringer (2 γ/ml) and α-(β-palmitoyl) - lysolecithin Ringer (10 γ/ml) for 5-10-20 and 40 min.*

The diagram shows the average values obtained from groups of 4 hearts and their standard error. Ord.: endocardiac K^+ in mEq/Kg of fresh tissue Abs.: time in min.

it appears therefore that the net loss of potassium begins only after 20 min from the commencement of the perfusion.

From the analysis of the mechanogram recorded in this series of experiments, the cardiotonic effect appears particularly evident in the initial period when no net transfer seems to occur. The net transfer seems, instead, to coincide with a subsequent phase when the mechanogram shows a tendency of the herats to enter a state of contracture, as seen in the previous series of exp. of the Figs. 3 and 4.

Discussion

Our results show that strophantin G as well as α-(β-palmitoil)-lysolecithin cause qualitatively similar effects on the potassium transfer and on the mechanical activity of the isolated, beating heart of rana esculenta. They increase temporarily the outflux of

Rb^{86}; this effect is particularly intense with the lysolecithin. Assuming, on the base of the chemical and physical analogy of the potassium and rubidium ions, that the behaviour of rubidium is similar to that of potassium, one may think that the increase seen in the outflux of Rb^{86} applies also to potassium.

From this fact alone, however, it would be difficult to state that such an increase is really due to an increased exchangeability of potassium. To make sure of this, one has to know whether the increase of the outflux rate corresponds to the same increase in the influx rate; if this is not the case, then our observation would correspond simply to a net loss potassium.

From the Figs. 3 and 4 one can see that the increase of the Rb outflux rate is very definite from the first minute after the addition of the drugs. However from Fig. 5 one can see that the net loss of potassium is a relatively late phenomenon which begins 20 min after the addition of the drugs. Therefore, in the first phase, when it is very definite, the increase of rate of the potassium outflux should be associated with a similar increase of rate of the influx. This enables one to suggest that, at least initially, the two drugs may cause a real increase of the cell exchangeability to potassium.

This increase of exchangeability might depend,in turn, on the reduction of the passive resistance of the membrane to potassium or on the mobilization of possible fractions of this ion provided with a lesser exchangeability with the outside. It seems that inside the cardiac cells there are two or more fractions of potassium which have different locations or different physico-chemical characteristic and are able to exchange with the external potassium at different rates (13, 15, 23). In this respect, our present results do not give any information; they only enable us to state surely that the two drugs under examination modify, at least initially, the cell exchangeability in the sense of increasing the rate of the potassium exchange between cell and ambient; this presumably occurs to the same extent in the two directions.

It has also been seen that the intracardiac content of potassium, in hearts treated for 40 min with the drugs under examination, is about 22% less than in the controls. It is well known that a strict relationship exists between the sodium and potassium content in a wide variety of cell types, so that when the concentration of the first increases that of the other decreases and vice versa: the normal concentration of these two electrolytes in the cell is apparently

determined by the efficiency of the active mechanism of Na-ion expulsion which may be linked to a partial or total exchange of potassium ions (cationic pump). The digitalis glycosides are able to inhibit the cation pump in a variety of substrates, such as erythrocytes (21, 22, 11, 4, 5, 10), heart (6), skeletal muscles (9), frog skin (12, 1), renal tubes (25).

The net loss of potassium caused on frog heart by both the drugs under examination could be the result of such an inhibition. However in a recent research carried out on frog skin (17), we were able to see that, while strophantin G inhibits the active transfer of the sodium ions, as already shown (12, 1), lysolecithin leaves it almost unchanged, except for an initial temporary increase. We would, then, prefer to think that the late loss of potassium seen in the heart treated with lysolecithin has a different mechanism from that due ot the strophantin G.

Considering now the relationship between mechanical activity and ionic exchange, one can see that the cardiotonic effect of the two drugs is evident particularly in the period when the potassium exchangeability increases but without an apparent net tranfer. In the subsequent period, when the net loss of potassium appears, the mechanogram reveals, instead, the tendency of the heart to enter a state of contracture. The inotropic effect of the two drugs, therefore, does not seem related to the intracardiac content of potassium. Instead, the secondary contracture is apparently related to the net loss of potassium but it is impossible to say whether the contracture is the result or the cause of the alteration of the intracellular ionic content.

In conclusion, the drugs under examination increase the contractile energy of the myocardium presumably through a mechanism completely independent from any alteration of the intracellular ionic content.

The contemporaneity of the cardiotonic effect and of the increased cell exchangeability to potassium might lead one to think that the modification of the mechanical activity is, related to this last phenomenon although it does not clarify the exact mechanism of action of the two drugs.

Summary

The results obtained from experiments on the isolated, beating heart of Rana Esculenta, at the same time, the effect produced by strophantin G and by a α-(β-palmitoil)-lysolecithin on the mechanical

activity, on the cell exchangeability and on the intracardiac content of potassium lead to the following conclusions:

The two drugs cause mechanical effects on the heart which are rather similar. The mechanical effect due to the two drugs, at the dosage we have used, includes two phases. The first, lasting 10-30 min, consists of an increase of the contractile energy; the second, subsequent to the first, consists of a state of reduction of activity which leads to systolization, of the heart and to an accentuation of the negative chronotropic effect. The first phase is associated to an increased cell exchangeability to potassium; the second is coincident with the appearance of the net loss of this ion.

REFERENCES

1. Aguggini, G. and Noseda, V. Depolarizing action of K – Strophantine and K – Strophantoside on isolated frog skin. *Experientia, 14*: 458, 1958.
2. Capraro, V., Marro, F. e Valzelli, G. L'effetto di una α-lisolecitina sull'attività meccanica e sulla cinetica di uscita del Rb^{86} nel cuore isolato di Rana esculenta. *Boll. Soc. ital. Biol. sper., 35*: 537-540, 1959.
3. Clark, A. J. The action of ions and lipoids upon the frog's heart. *J. Physiol., 47*: 66-107, 1913.
4. Glynn, I. M. The action of cardiac glycosides on red cells. *J. Physiol., 128*: 56-57 P, 1955.
5. Glynn, I. M. The action of cardiac glycosides on sodium and potassium moviments in human red cells. *J. Physiol., 136*: 148-173, 1957.
6. Hajdu, S. and Leonard, E. The cellular basis of cardiac glycoside action. *Pharmacol. Rev., 11*: 173-209, 1959.
7. Hajdu, S. and Szent-Györgyi, A. Action of DOC and serum on the frog heart. *Amer. J. Physiol., 168*: 159-170, 1952.
8. Hajdu, S., Weiss, H. and Elwood, T. The isolation of a cardiac active principle from mammalian tissue. *J. Pharmacol. 120*: 99-113, 1957.
9. Harris, E. Y. Permeation and diffusion of K ions in frog muscle. *J. gen. Physiol., 41*: 169-195, 1957.
10. Harris, E. Y. and Prankerd T. A. Y. The rate of sodium extrusion from human erythrocytes. *J. Physiol., 121*: 470-486, 1953.
11. Joyce C. R. B. and Weatherall M. Cardiac glycosides and the potassium exchange of human erythrocytes. *J. Physiol., 127*: 33 P, 1955.
12. Koefoed-Yohnsen, V. The effect of g-strophantin (ouabain) on the active transport of sodium through the isolated frog skin. *Acta physiol. scand., 42*: suppl. 145, 87-88, 1957.
13. Marro, F. e Capraro, V. L'effetto dell' α-eparina sullo scambio del Rb^{86} nel cuore isolato di Rana esculenta. *Boll. Soc. ital. Biol. sper., 34*: 1702-1706, 1958.
14. Marro, F., Lodi, M. P. e Bartoli, E. Analisi degli effetti esercitati dalla g-strofantina e da una α-(β-palmitoil)-lisolecitina sul contenuto potassico del cuore isolato e pulsante di Rana esculenta. *Boll. Soc. ital. Biol. sper., 35*: 1813-1815, 1959.
15. Marro, F., Pesente, L. e Capraro, V. L'effetto di soluzioni ipocalciche sullo scambio del Rb^{86} nel cuore isolato di Rana esculenta. *Boll. Soc. ital. Biol. sper., 35*: 171-173, 1959.
16. Marro, F., Pesente, L. e Capraro, V. L'effetto dell' α-eparina sullo scambio del potassio con il Rb^{86}, nel cuore isolato e pulsante di Rana esculenta. *Boll. Soc. ital. Biol. sper., 35*: 540-543, 1959.

17. Marro, F., Pesente, L. e Capraro, V. Effetti della g-strofantina e di una α-(β-palmitoil)-lisolecitina sul trasporto di sodio e sul passaggio di acqua a livello della pelle isolata di Rana esculenta. *Boll. Soc. ital. Biol. sper.*, *36*, 1843-1847, 1960.
18. Pinotti, O. L'influenza del fegato sul metabolismo energetico del cuore. *Arch. Fisiol.*, *42*: 170-182, 1942.
19. Rein, E. Physiologische Beziehungen zwischen der Leber und dem Energiestoffwechsel des Herzens. *Klin. Wschr.*, *21*: 873-877, 1942.
20. Scarinci, V., Parenti, M. A., Cantone, A. and Ravazzoni, C. Biochemical and pharmacological observations on a α-(β-palmitoyl)-lysolecithin. *Arch. int. Pharmacodyn.*, *128*: 472-480, 1960.
21. Schatzmann, H. J. Herzglykoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrocytenmembran. *Helv. physiol. Acta*, *11*: 346-354, 1953.
22. Schatzmann, H. J. Die Wirkung von Desoxycorticosteron auf den aktiven Kationenaustausch an Rattenblutzellen. *Experientia*, 10: 189-190, 1954.
23. Schreiber, S. S. Potassium and sodium exchange in the working frog heart. Effects of overwork, external concentrations of potassium and ouabain. *Amer. J. Physiol.*, *185*: 337-347, 1956.
24. Stutz, M., Feigelson, E., Emerson, J. and Bing, R. J. The effect of digitalis on the mechanical and electrical activity of extracted and non extracted heart muscle preparations. *Circulation Res.*, *2*: 555-564, 1954.
25. Whittembury, G. Ion and water transport in the proximal tubules of the kidney of Necturus maculosus. *J. gen. Physiol.*, *43*: 43-56, 1960.

THE REPTILIAN FOREBRAIN

I. The Olfactory Pathways and Cortical Areas in the Turtle [1]

F. ORREGO

Washington University, Department of Zoology
Washington University, School of Medicine, Division of Neurology
St. Louis (Mo.), U. S. A.

Introduction

"From the dawn of interest in the minute structure of the human brain, it was recognized that the simpler brains of lower vertebrates present the fundamental features of the human brain without the numberless complications which obscure these fundamentals in higher animals." (16, p. 4).

With this sentence, Herrick explains the scope of his investigation of the brain of the Tiger Salamander, which started in 1910 and continued with no interruption for the following 40 years. The results of this analysis are collected in his books: *The Brain of the Tiger Salamander* and *The Evolution of Human Nature* (16, 17). In both books, the author emphasizes the need for integrating the morphological analysis with functional investigations of brains of lower vertebrates. He expresses the hope that "when the facts about the sequence of the evolution of cortical structure and function are colligated with experimental studies of behavioral capacities of the animal in question, we shall have a secure foundation upon which to build a sound comparative psychology, and this, in turn, will clarify much that is now obscure in human experience" (16, p. 104).

An extensive literature on brain structures from lower to higher vertebrates is presented in the classic books by Cajal (7), Kappers (23), Kappers, Huber and Crosby (22), Herrick (14, 16, 17), Papez (26),

[1] This work has been supported by a grant from the Public Health Service, Number B-1602.

Johnston (18), and Beccari (5). They are written from a comparative neurological and evolutionary viewpoint, but none of them makes use of the electrophysiological analysis of these structures. On the other hand, in the few instances in which this technique was applied to submammalian forms, the comparative aspect of the problem was neglected.

The only investigations on turtles to be mentioned in this connection are the ones by Johnston (20) and by Tuge and Yazaki (29) and the more recent ones by Bremer, Dow, and Moruzzi (6). Johnston, using the technique of electrical stimulation, concluded that motor activity can be evoked from discrete areas of the cerebral cortex. Bremer, Dow, and Moruzzi, using more precise techniques, came instead to the conclusion that the activity was due to spreading of the stimulus to lower centers. They also analyzed the spontaneous activity of the cerebral cortex in turtles. Other authors investigated the motor activity evoked by stimulation of the cortex in alligators (3) and in the lizard (29).

One of the most controversial arguments in comparative neurology concerns the use of the term, " cortex ", to define the organization of the dorsal pallium. If the term is to be applied only to a pallium organized in distinct cellular layers, then it would not apply to forms lower than the reptiles (16). If, instead, the term should be extended to include a pallium which receives thalamic radiations, then one should agree with Johnston that selachians already have a very primitive cortex (21).

This problem could be settled only by integrating the morphological criteria with functional analysis of the cerebral pallium. In the present investigation, we selected reptiles, and among reptiles, turtles, since they are considered the most generalized living reptiles, at least as far as brain structures are concerned (14). These forms possess, according to Herrick, the most primitive type of cerebral cortex. However, Johnston considers the cortex of reptiles as already fairly advanced in evolution. " The neopallium in reptiles is by no means in a primordial stage ", he writes, " but has already a broad extent with localization of function " (21, p. 184). Our functional analysis is in agreement with this statement. As will be shown in the discussion of the first paper, evidence was found that the cerebral cortex in the turtle already has a well-defined topographic organization.

Besides presenting a favorable material in which to investigate

this phylogenetical problem, the cerebral cortex of the turtle offesr another advantage. The almost schematic geometrical organization of fibre tracts and of their synaptic endings on dendrites and on cell bodies in this cerebral cortex offers ideal material for the study of some problems of general electrophysiology in the central nervous system.

The results to be presented in the following papers represent the first part of the research program now in progress. Throughout all the investigation, an attempt was made to integrate the morphological and the electrophysiological methods, using the latter also as a tool to trace fibre tracts and to check the morphological data.

The first paper deals with the structural organization of the turtle's olfactory bulb and cerebral cortex. In the following paper, we report on the characteristics of the response of nerve cells in the olfactory bulb to olfactory nerve stimulation. The third paper is devoted to the analysis of the functional interrelation of the two olfactory bulbs and of the cerebral cortex of the two hemispheres. In the fourth paper, a preliminary analysis of the electrophysiological organization of the cerebral cortex is presented.

In this paper a brief outline of the morphological organization of the olfactory bulbs of the cortex and of the main fibre tracts connecting the olfactory bulbs with the homolateral and contralateral cerebral hemispheres will be presented. It is necessary to emphasize that no attempt was made to cover all the aspects of the organization of the olfactory bulbs and of the cerebral cortex; only those structures will be described which are pertinent to the electrophysiological analysis to be presented in the following papers.

Extensive literature is available on the reptilian brain. Here, only the important contributions by Herrick (13), Ramon (27, 28), Johnston (19), Crosby (8), and Gamble (10) will be mentioned. A recent review on this topic by Goldby and Gamble gathered the information scattered in different journals in a comprehensive ar ticle (12).

While these papers cover the main features of the cerebral hemispheres in reptiles, other investigations were devoted to the analysis of the olfactory system from a comparative point of view. An extensive study by Crosby and Humphrey (9) considered the evolutionary aspect of the olfactory bulbs. In 1953 Allison wrote a very valuable review of the olfactory system in all vertebrates (2). We integrated the available information with observations on our own material. Furthermore, as already mentioned, we made use of the electrophysiological technique with the aim of tracing fibre

tracts. The characteristics of the electrical activity and the functional interaction of different components of the olfactory system and of the cerebral cortex are not considered in this paper.

Methods

The animal used in this and in the following investigations was the turtle (*pseudemis scripta elegans*) 25 to 30 cm long. Early in the project ether was administered before beginning operations, but since there was no sign of an anesthetic effect, its use was discontinued. The olfactory nerves, the olfactory bulbs, and the cerebral hemispheres were exposed homo- or bilaterally by opening the skull with a dental drill. Coagulating cotton (oxycel) was used to stop small hemorrhages, and a high frequency coagulator was used for large vessels. In turtles a rather thick vascularized membrane covers the brain: it is possibly the homologue of the mammalian dura. The removal of this membrane leaves a very thin nonvascularized membrane, possibly the homologue of the mammalian arachnoid which extends also on the surface of the olfactory nerves. It was carefully torn apart with fine forceps. The space between this and the surface of the brain is filled with the cerebrospinal fluid. Care was used not to damage the large midline blood vessels, since even minor injuries would result in impairment of the circulation of the hemispheres. In a first group of experiments, the immobilization of the head was accomplished by total extirpation of the powerful head and neck muscles and of the soft tissues surrounding them. The brain remained connected with the body only through the blood vessels and the spinal cord which was kept intact. This very laborious and time-consuming procedure was replaced in a second larger group of experiments by light curarization. The curare (flaxedil) was administered in the jugular vein through a permanent plastic cannule inserted in the caudal stump of the blood vessel. The dose necessary for immobilization was 0.5 ml of flaxedil diluted 1:10. During the recording period, small amounts of this solution were injected to keep the animal under light curarization. After cannulation of the jugular vein, the trachea was sectioned and two plastic tubes were inserted all the way to the lungs. Oxygen and CO_2 (95-5%) were insufflated continuously into the lungs. The recording was done with teflon-insulated stainless steel wire electrodes. When monopolar recording was used, the surface recording electrode was a 40 μ wire; when bipolar recording was used, the deep lead was a 20 μ wire ground to a very sharp tip. The same 20 μ wire was used for fractional recordings or deep monopolar recording. The stimulating electrodes consisted of two or three 20 μ wires cemented together and ground to a sharp tip (the third lead was connected to a shock balancing circuit).

The recording electrodes were connected through very short leads to a cathode follower-type probe, and the activity amplified conventionally through a Grass condenser coupled preamplifier (pushpull input half wave amplitude 0.1 cycle for low frequencies and 30 kilocycles for high frequencies) and displayed on a Tektronix Model 502 oscilloscope. In some experiments, the brain was kept under a pool of mineral oil maintained at a constant level by automatic dropping. In other experiments, we used a pool of oxygenated Ringer-glucose through the same dropping procedure. Since this second technique favored a longer survival of the preparation, it was then used in most of the experiments. The localization of the deep electrode was identified by an electrolytic iron deposit and subsequent stain with the ferrocyanide technique.

The histological analysis of the olfactory and of the cerebral cortical structures was performed on a large number of brains dissected and stained with the silver and other standard histological techniques. The silver techniques used were: the Cajal (Chloralhydrate and pyridine modifications of the

classic method), the Bodian, and the rapid Golgi-Cox methods. Brains stained with hematoxylin in toto proved to be very useful, not only for staining nerve cells, but also for tracing of fibre tracts. The brains were sectioned serially at 20 μ with the exception of the Golgi stained material which was sectioned at 80 μ. The sections were cut in different preparations along the three conventional planes: transverse, sagittal, and frontal.

Results

I. – *Anatomical observations.*

The telencephalon of the turtle is roughly pear-shaped. At its anterior end is the olfactory nerve which runs a distance of approximately 6 mm between the olfactory mucosa and the olfactory bulb. The length of the olfactory nerve in the turtle makes it especially convenient for the study of the physiological input to the olfactory bulb. The telencephalon has an over-all length of approximately 15 mm of which one-third corresponds to the comparatively large olfactory bulb and two-thirds to the hemispheres (Fig. 1).

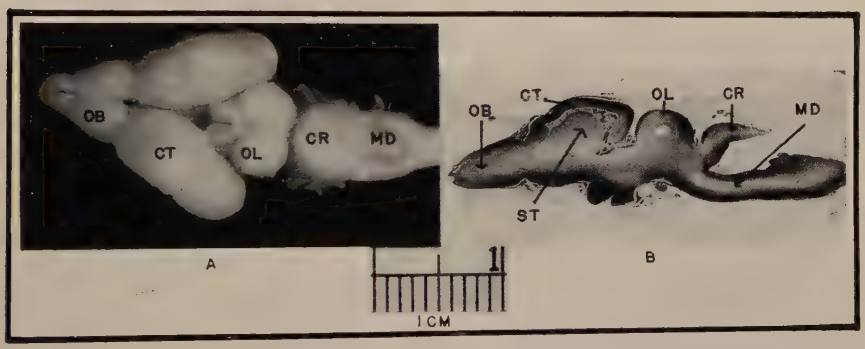


Fig. 1. – *To the left, a picture of the whole brain of a 10 inch turtle.* To the right, a parasagittal section of the same brain stained with silver technique. Lower scale, one centimeter: OB, olfactory bulb; CT, cortex; OL, optic lobes; CR, cerebellum; MD, medulla; ST, striatum.

Johnston (19) gave a very accurate description of the turtle brain which provided the background for the following investigations including ours. In the present study, our interest will focus on the olfactory bulb, olfactory pathway, and the structural organization of the cortex.

A brief description of the principal structures as they appear in silver and in the hematoxylin stained material will be given here. Only those structures which were analyzed with electrophysiological methods will be considered. Fig. 1 *B* shows a sagittal section of a turtle brain stained in toto with the pyridine silver technique.

1) *Olfactory nerve.* — The axons of the olfactory nerve are extremely fine. The spectrum of diameter shows a peak in the region of 0.25 μ with very few fibres over one μ in diameter (Bishop, personal communication).

2) *Olfactory bulb.* — In the olfactory bulb, the following fibres and cell layers are present (Figs. 2, 3, 4).

1. The layer of afferent olfactory fibres densely packed at the surface of the bulb and apparently not organized in well defined bundles.

2. The glomerular layer which consists of the arborization of olfactory nerve fibres and the dendritic tufts of the mitral cells. In this layer, there are a fairly large number of small nerve cells known as intra- and interglomerular cells.

3. The external plexiform layer formed by the apical dendrites of the mitral cells. The basal dendrites of the same cells make a contribution to this layer as they ascend toward the surface and

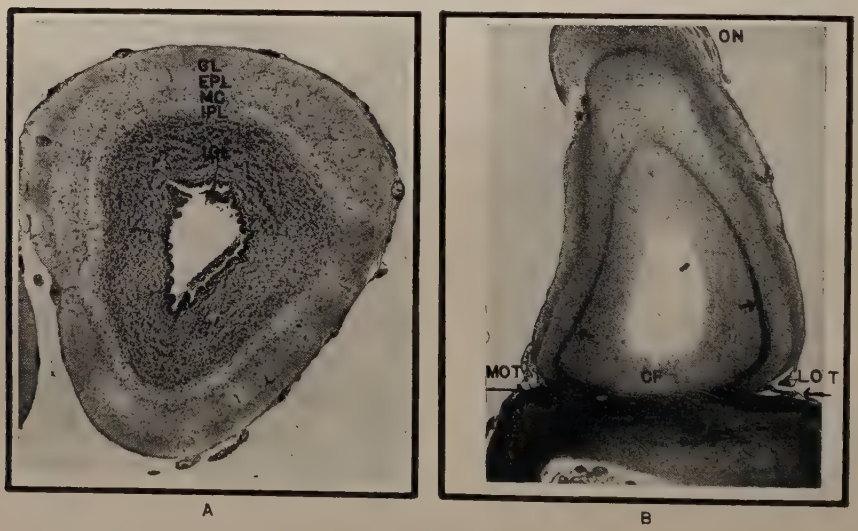


Fig. 2. – *To the left, transversal section of the olfactory bulb (toluidin stain).*

GL, glomerular layer; EPL, external plexiform layer; MC, mitral cell layer; IPL, internal plexiform layer; IGL, internal granular layer.

To the right, a frontal section of the olfactory bulb (Cajal technique). ON, olfactory nerve; MOT, medial olfactory tract; LOT, lateral olfactory tract; CF, crossing fibres in the back of the bulb coming from mitral cells in the lateral and medial aspects of the bulb. Arrow points to the fibre bundles consisting of the axons of the mitral cells.

Fig. 3. – Frontal section of the olfactory bulb (Cajal technique). ON, olfactory nerve fibres; GL, glomerular layer; EPL, external plexiform layer; MC, mitral cell layer; IPL, internal plexiform layer; IGL, internal granular layer. The arrow points to one mitral cell of the " associative type ".

end in close proximity of the glomeruli (Fig. 4). Dendrites of granular cells also branch in this layer.

4. The layer of the mitral cells which do not present in a turtle the rigorous distribution in one compact row as in mammals. They are, in fact, organized in a rather irregular fashion and rather sparsely distributed in the layer. The basal dendrites may, as already mentioned, become ascendent, or they run horizontally for a length which in some instances reaches 2 mm (Fig. 3).

The mitral cells vary considerably in shape and size from 10 to 40 μ. Most of the small mitral cells give origin to the long horizontal dendrites mentioned above. Dendrites from the internal granular layer extend in this layer.

5. The internal plexiform layer consists of densely packed nerve fibres deeply stained with silver. They are the axons of the mitral cell sand of other more deeply located neurons.

Crosby (8) described these deeply located cells in the alligator and designated them as " goblet cells ". The same type of cell is present in the turtle. They are located in the internal granular

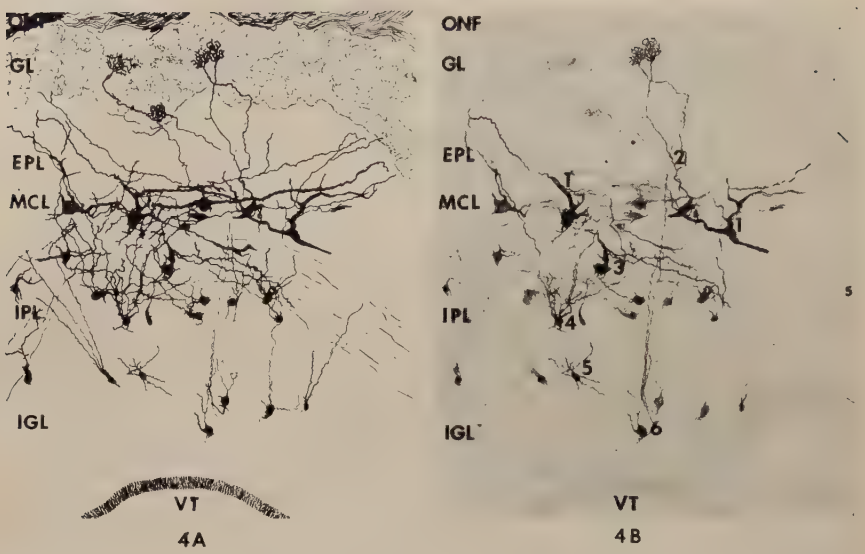


Fig. 4. – *A*: camera lucida drawing resulting from superimposing of six serial transversal sections of the olfactory bulb (Golgi technique). ONF, olfactory nerve fibres; GL, glomerulars; EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; IGL, internal granular layer; VT, ventricle.

B: some cells are heavily stained to show the main branching characteristics, and interconnections of some of the neurons of each group: 1 and 1', fine axons coming from the deeper layers and running in close relationship with the basic dendrites of the mitral cells; 2, ascending dendrites of a mitral cell giving origin to two branches that connect to two different glomeruli; 3, cell giving origin to a long axon that runs together with the axon of the mitral cells in the internal plexiform layer. The axon runs in a cephalocaudal direction and, therefore, only the initial part of it is seen in the drawing; 4 and 6, granular cells with very long processes extending, almost to the glomeruli; 5, Golgi 2 type cell.

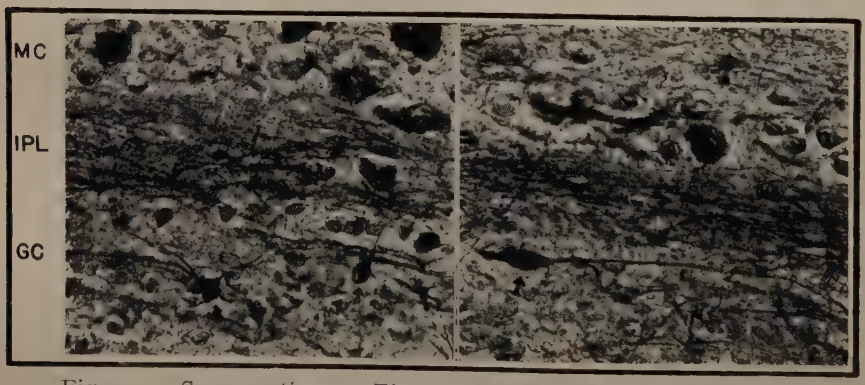


Fig. 5. – *Same section as Fig. 3 at higher magnification.*

MC, mitral cells; IPL, internal plexiform layer; GC, granular cells. Arrow points to granular cells sending their axons to the internal plexiform layer.

layer; the axons run in the internal plexiform layer together with the axons of the mitral cells and take a horizontal direction while the axons of other granular cells are, instead, ascendent (Fig. 5). The possibility suggested by Allison (2) that they are equivalent to mammalian tuft cells is supported by observations to be reported in a following paper. In this layer, the recurrent axons of the mitral cells which end in the following layer are seen.

6. The internal granular layer consists of the granular cells, the goblet cells, and their dendritic arborizations.

3) *The olfactory tracts.* — The axons of the mitral cells which give origin to the heavily stained internal plexiform layer segregate into two well-defined lateral and medial fibre tracts at the posterior end of the olfactory bulb. Mitral cells located laterally contribute to the medial fibre tract and, conversely, medially-located mitral cells send their axons in the lateral tract. The crossing of olfactory nerve fibres in the area between the olfactory bulb and cortex is clearly evident in our material (Fig. 2 *B*). The lateral tract sends fibres to the anterior olfactory nucleus, to the nucleus of the olfactory tract, to the olfactory tubercle, and to the striatum. The main stream of these fibres, however, runs along the surface of the cortex. Its termination in the piriform cortex and in the posterior part of the general cortex was ascertained by electrophysiological technique (see below). The medial olfactory tract runs on the surface of the hippocampal and septal areas. The nerve fibres were traced to the hippocampal commissure. It is most likely that they cross in this commissure (see paper II).

4) *The cortex.* — It is generally agreed that the reptilian cortex consists of three main areas. Medially, the hippocampus; dorsally, the general cortex; and laterally, the piriform cortex (Fig. 6). According to Johnston (19), the hippocampus expands laterally on the anterior part of the pallium and joins the anterior part of the piriform cortex.

The thickness of the cortex in fresh material varies between 200 and 500 μ. The lateral ventral part of the piriform cortex lies directly on the striatum. The other cortical areas are separated from the underlying structures by the ventricle. The piriform and general cortex are separated from each other in the anterior part of the " pallial thickening " (19). The limits between the hippocampal and general cortex are not clearly defined.

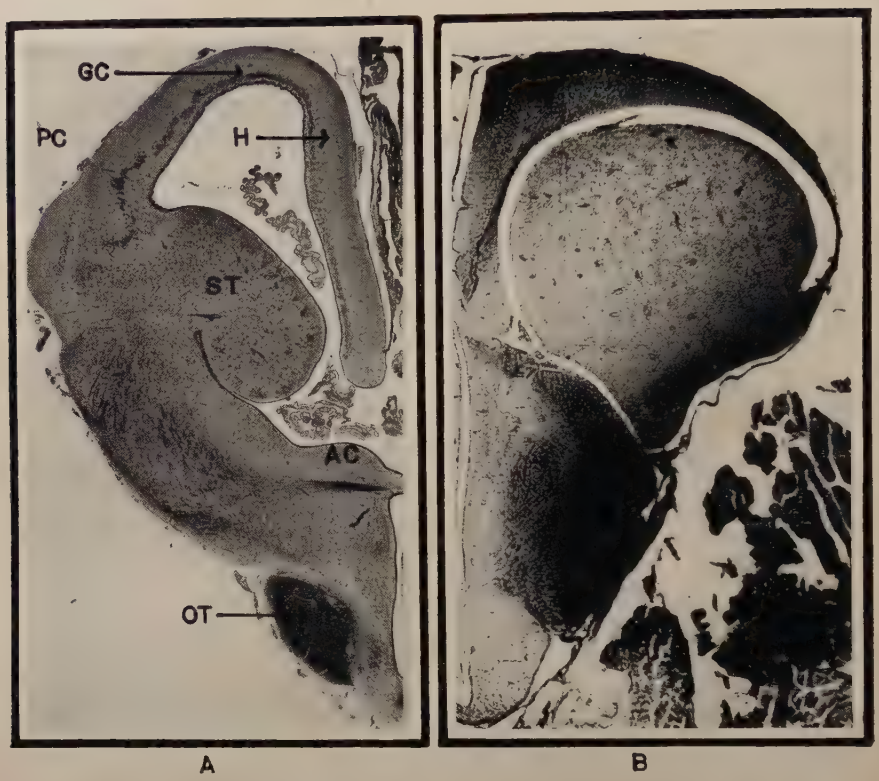


Fig. 6. – *A*: transverse section of turtle cerebral hemispheres. H, hippocampus; GC, general cortex; PC, piriform cortex; OT, olfactory tract; AC, anterior commissure; ST, striatum. Above the anterior commissure, the hippocampal commissure fibres weakly stained.

B: transversal section of hemisphere of *anolis carolinensis*, a microsomatic reptile. Notice the almost complete absence of the piriform cortex and the well-developed hippocampus and striatum.

The histological structure is similar throughout the cortex. The cells are situated in a deep layer two or three cells thick and only a very thin fibre layer separates them from the underlying ventricle.

The dendritic arborizations of these neurons are clearly seen in Golgi stained material (Fig. 7 *B*). They reach the most superficial layer of the cortex.

Two systems of nerve fibres are seen in silver-stained material (pyridine Cajal technique): one runs along the surface and the other runs deep in contact with the nerve cell bodies (Fig. 7 *A*). In the piriform cortex, the superficial fibre system consists chiefly of the lateral olfactory tract. A similar superficial fibre tract is present

in other pallial areas. Evidence will be presented that this system in formed by sensory afferent fibres of different modalities discharging in these cortical areas. Morphological, as well as electrophysiological,

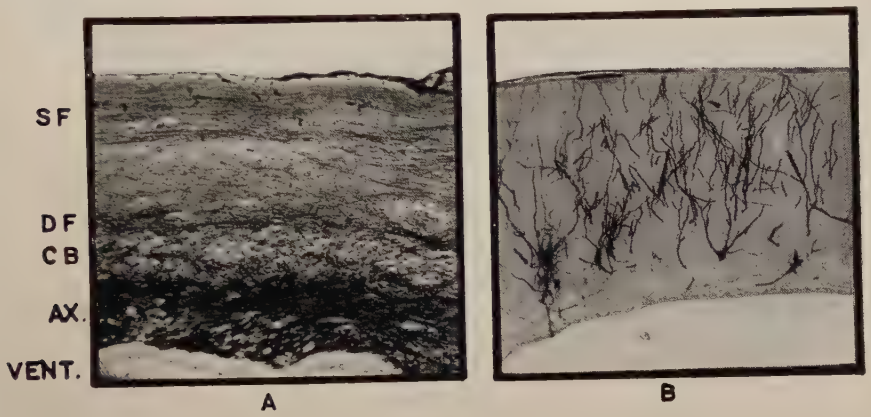


Fig. 7. – *A*: transversal section through the piriform cortex (Cajal technique). SF, superficial fibres; DF, deep fibres; CB, layer of cell bodies; AX, axons of the cells; VENT, ventricle.
B: similar section shows the dendritic arborizations of the cortical cells reaching the surface of the cortex (Golgi technique).

analysis suggests that the deep fibre tract is an associative system. Fibres originating in the deep granular layer of the olfactory bulb also contribute to this system. This will be discussed in detail in a following paper.

II. – *Electrophysiological investigations.*

The method of evoked potentials was used to trace fibre tracts and in this way to integrate the morphological data presented above. No attemp is made here to analyze the characteristics of the evoked response since is the main object of the followoving papers.

1) *Response of the olfactory bulb to eletrical simulation in the olfactory nerve.* — Stimulation of the dorsal surface of the nerve with a 20 μ electrode gives origin to a surface-native potential in the dorsal surface of the olfactory bulb. The response becomes

diphasic if the stimulating electrode is pushed deeper into the nerve, inverts, and becomes surface-positive by an even more deep penetration of the stimulating electrode in the nerve.

Stimulation of the dorsal surface of the nerve and recording in the ventral surfage of the bulb results in a surface-positive response while stimulation of the deep fibres of the nerve evokes a surface-negative response from the ventral aspect of the bulb.

The mirror image of the electrical activity strongly suggests that two distinct fibre bundles originating in the dorsal and ventral aspect of the olfactory mucosa and projecting to the dorsal and wentral part of the olfactory bulb, respectively, are being dealt with here.

The exceedingly small size of the olfactory nerve fibres is reflected in the speed of conduction which was found to be of the order of 0.15 m/sec.

2) *Cortical response to olfactory nerve stimulation.* — Electrical stimulation of the nerve evokes a surface-negative response in the piriform cortex and in the posterior part of the general cortex. If monopolar recording is used, a small surface-positive response is recorded in the dorsal edge of the hippocampus and in a large part of the general cortex. No response is recorded in these areas when the bipolar recording is used. Most of the hippocampus and of the septal region were silent to olfactory nerve stimulation. It must, however, be pointed out that in order to expose these areas, some damage to the blood supply is unavoidable; although it is unlikely that the lack of response in this area is due to circulatory damage. The posterior pole of the cortex was likewise silent. The diagrams in Figs. 8, 9 show the cortical area which responds to olfactory stimulation. Briefly, the characteristics of the evoked response in Fig. 8 are as follows:

Area A: evoked potentials exhibit a rather sharp ascending and descending phase and are relatively short in duration if compared to the activity of other olfactory cortical areas. The characteristics of the response and the topographical location of the olfactory tract in this region suggest that the recorded activity is mainly due to the olfactory tract itself. Since the nucleus of the olfactory tract and the anterior olfactory nucleus are closely associated with the fibre tract, the response might also reflect the activity of these cells.

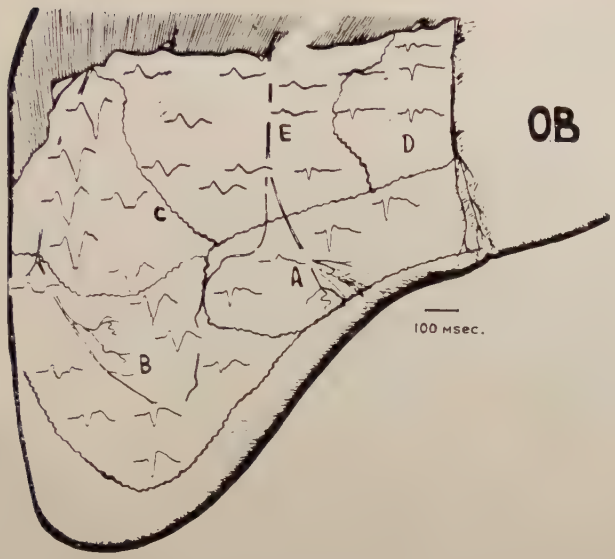


Fig. 8. – *Mapping of the responsein the cortex to olfactory nerve stimulation. The characteristics of the potential in each one of the marked areas are described in the text.*

OB, olfactory bulb. Upward deflection indicates surface positivity.

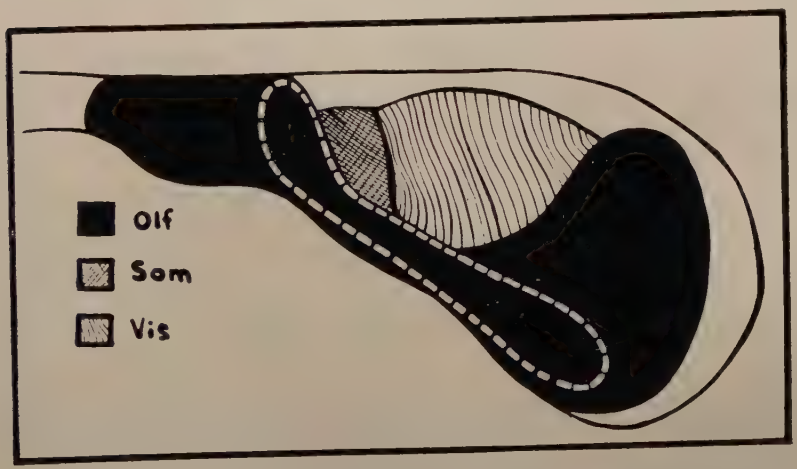


Fig. 9. – *Schematic drawing of the projection of the different sensory modalities over the olfactory bulb and cortex.*

Olf, olfactory areas; Som, sensory areas; Vis, visual areas. For explanation see text. Broken line: area described by Johnston as a motor cortex.

Area B: this area corresponds roughly to the piriform cortex. The evoked potentials show considerably larger amplitude and duration. These characteristics of the response, as well as the excitability cycle (see following paper), suggest that we are dealing with a dendritic response.

Area C: this area occupies the posterior part of the general cortex. Here the potentials are similar to those described in Area B, but the form is much more complex.

Area D: this area corresponds roughly to the dorsal anterior extension of the hippocampus described by Johnston (19). Activity is recorded only when the ventral fibres of the olfactory nerve are stimulated.

Area E: the characteristics of the response which can only be recorded monopolarly, as mentioned above, suggest two alternatives: either the activity is recorded at a distance or it represents a secondary response mediated through cortico-cortical associative fibres in the deep system of the cortex.

3) *Cortical response to optic nerve stimulation.* — While the projection of the olfactory nerve to the cerebral cortex in reptiles is a well-known and generally accepted fact, the direct projection of other systems such as the visual system, has never been prospected.

In the following, the topography of the visual area and the characteristics of the response will be briefly presented.

Electrical stimulation of the optic nerve evokes activity in a large area of the general cortex labelled Area E in Fig. 8. The possibility that the response arises in the underlying neural structures is ruled out by the following facts: *a*) the response is surface-negative; *b*) the polarity reverses in the deep layers of the cortex when fractional leads are used; *c*) a superficial incision in the lateral cortex abolishes the response; in these experiments the histological control showed that deep structures had not been injured. These results give evidence that we are dealing with a local response; however, they do not prove that the cortical area is a projection area for the optical pathways.

The following observations strongly suggest that we are actually dealing with a projection and not with an association area.

Electrical stimulation of the optic nerve or illumination of the retina result in a response characterized by four well-defined spikes

in the nerve and optic lobes. Spikes with the same delay-constant are recorded in Area E of the cortex (Fig. 10).

It is of interest to notice that the characteristics of the potential in this area are different in the anterior and posterior sectors. The

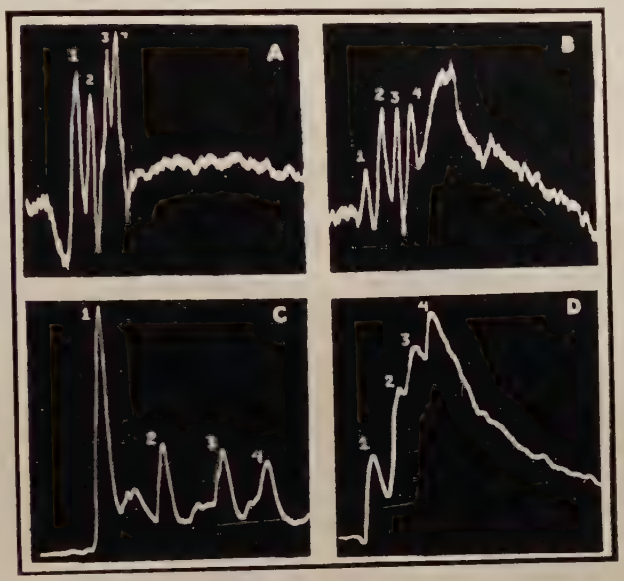


Fig. 10. – Response to a short flash to the retina in A, optic nerve; B, optic lobes; C, visual cortex. As explained in text the "delay constant" between the four mani spikes is the same in all records. D, response in the visual cortex to electrical stimulation of the optic nerve. An upward deflection indicates surface-negativity for optic lobes and cortex.

activity evoked in the latter is shorter in duration and also less complex than in the former. A similar difference in the response was observed in Areas C and D to olfactory nerve stimulation.

4) *Cortical response to stimulation of the dorsal funiculi.* — Electrical stimulation of the dorsal funiculi at the cervical level of the spinal cord results in activity in front of Area E (Figs. 8 and 9). Since these results were obtained with monopolar recording, the possibility cannot be entirely ruled out that this activity represents recording at a distance from deeper structures. However, the fact that the response is surface-negative does not favor this possibility.

5) *Motor response to cortical stimulation.* — The question has been raised many times whether some area of the cerebral cortex in reptiles has motor function. In unanesthetized animals, stimulation of the piriform cortex and associated olfactory areas resulted consistently in widespread motility. Repetitive stimulation proved to

be more effective than simple shocks. Motility was also observed when the olfactory nerves or the olfactory bulbs were stimulated. The significance of these results will be discussed below.

6) *Contralateral cortical responses to olfactory nerve stimulation.* — The contralateral olfactory bulb and the piriform and associated

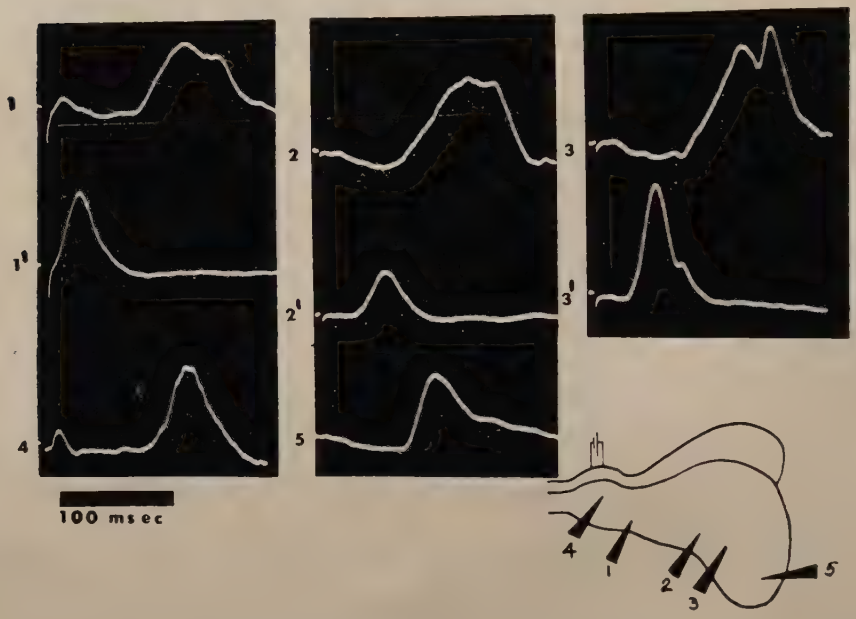


Fig. 11. – *Activity at different points of the surface of the brain evoked by stimulation of the olfactory bulb. The points of recording are marked in the insert.*

Records 1, 2, 3, 4, 5, activity evoked in the contralateral side. Records 1', 2', 3', activity in the homolateral side in points symmetrical to 1, 2 and 3. Monopolar recording with a surface lead.

olfactory cortical areas were tested. The following results were obtained (Fig. 11): a) *olfactory bulb*: a slow potential is evoked by stimulation of the olfactory nerve and more readily by stimulation of the opposite olfactory bulb; b) *piriform cortex*: the response is rather complex and consists of at least two different waves each with different characteristics. Evidence that they are related to independent fibre tracts will be presented in a following paper;

c) *olfactory Area E in the general cortex*: the response evoked in this area is similar to the one described in the homolateral Area E.

7) *Interhemispheric cortical relations.* — Stimulation of the piriform cortex of one side gives origin to evoked activity in symmetrical

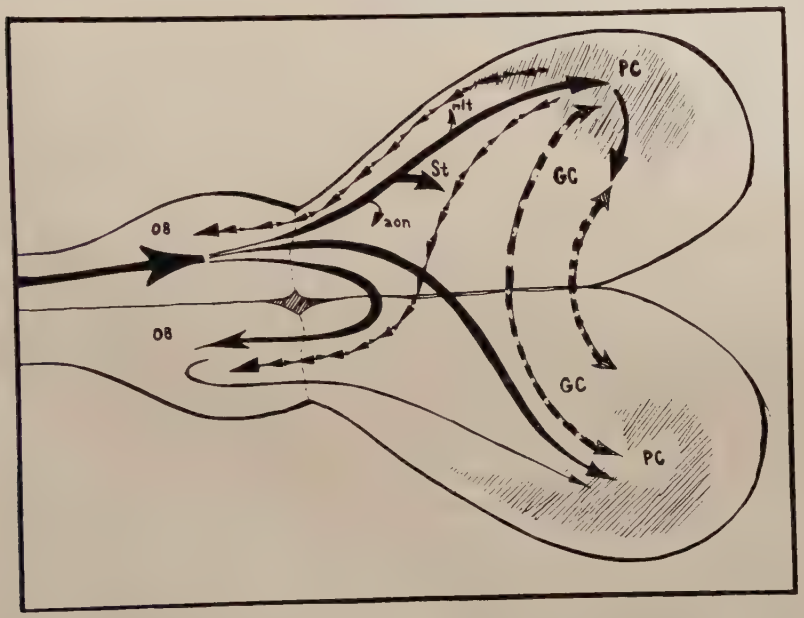


Fig. 12. – *Diagrammatic representation of olfactory pathways and interhemispheric connections based on electrophysiological recording.*

Heavy lines, main olfactory pathways. Broken arrows, efferent pathways from cortex to bulbs. Broken line, interhemispheric cortical connections. OB, olfactory bulb; AON, anterior olfactory nucleus; ST, striatum; NLT, nucleus of the lateral olfactory tract; PC, piriform cortex; GC, general cortex.

points of the opposite hemisphere. Stimulation of the lateral part of the general cortex also gives origin to activity in the general cortex of the opposite side.

8) *Cortico-bulbar relations.* — Stimulation of the piriform cortex evoked an electrical response in the homolateral and contralateral olfactory bulbs. A physiological analysis of this response strongly suggests the existence of an efferent system overshadowed by the antidromic response (see paper III).

Discussion

The aim of this paper is to provide a morphological background for a study of the functional organization of the olfactory system and of the cerebral cortex in the turtle. The results will be commented upon briefly since most of them will be considered again in the following papers after presentation of the electrophysiological analysis of each one of these structures.

It has been an object of discussion whether or not the olfactory epithelium projects to distinct areas in the olfactory bulb. Adrian (1), using electrophysiological techniques, was able to demonstrate that the oral extremity of the olfactory mucosa projects to the anterior part of the bulb while the caudal part of the mucosa projects to the posterior part of the olfactory bulb. Le Gros Clark (24, 25), using the method of retrograde degeneration, found evidence of a dorso-ventral segregation. Both investigations were performed in the rabbit. We also found evidence of a similar spatial localization in the olfactory projections in the turtle. The electrical stimulation of the olfactory nerve showed the existence of the two distinct fibre bundles; one, originating in the dorsal part of the nasal mucosa, projects to the dorsal part of the bulb; the other, originating in the ventral part of the same mucosa, projects to the ventral part of the olfactory bulb. The dorsal bundle may include the vomero-nasal nerve described by Crosby and Humphrey (9) as projecting to the accessory olfactory bulb that occupies the dorsal portion of the bulb. Our morphological and electrophysiological analysis did not show differences between this and the remaining part of the bulb. The evoked potential technique indicates that the ventral bundle projects mainly to the anterior part of the hippocampus while the dorsal bundle projects only to the piriform cortex. Herrick and other authors describe two well-defined components in the olfactory system: the olfacto-visceral relaying the hippocampus to the hypothalamus and the olfacto-somatic relaying the lateral wall of the hemisphere to the motor somatic nuclei of the mesencephalon. Our findings, as reported above, suggest that such segregation is already present in the olfactory nerve. As mentioned above, stimulation of the olfactory nerve evokes activity only in the small dorsal portion of the hippocampus. These results are not in agreement with the concept that the hippocampus subserves mainly the olfactory func-

tion in lower vertebrates. It remains for further investigations to establish the functional connections of the larger remaining part of the hippocampus. On the other hand, the olfactory function of the piriform cortex receives further confirmation from our analysis with electrophysiological techniques. In line with this finding is the observation that in a microsomatic reptile, the American chameleon (*Anolis Carolinensis*), the piriform cortex is altogether absent (Fig. 6 *A*, *B*) and the hippocampus instead is well developed.

Our results give evidence for a projection from each olfactory nerve through the olfactory bulb to the homolateral cerebral cortex and to the contralateral olfactory bulb and its cerebral cortex. The existence of a commissural system interconnecting the cerebral cortex of the two hemispheres was established with electrophysiological technique.

Another aspect of the results reported in this investigation deserves comment. The electrophysiological analysis gave evidence for the presence in the dorsal cortex of discrete areas of projection for visual and olfactory modalities. Furthermore, the stimulation of the spinal cord also evoked a response in a discrete area of the dorsal cortex; the response was well localized even to maximal stimulation. These results are indicative of the existence of a somatic cortex. The above findings raise the question of whether the term "general cortex" in reptiles is appropriate. We were unable to demonstrate the existence of a discrete motor cortical area in turtles. These results are in agreement with previous findings by Bremer, Dow and Moruzzi (6).

These authors interpreted the motility resulting from stimulation of the cortex as due to spreading of the stimulus to lower centers. This mechanism may certainly account for the motor reaction after stimulation of cortical areas covering the striatum. We observed, however, motility also following stimulation of the olfactory bulb and even after stimulation of the olfactory nerve in non-anesthetized animals. The generalized and irregular character of these movements suggest a possible reflex mechanism.

Summary

The olfactory system in the turtle was investigated by combined morphological and electrophysiological techniques. It was found that each olfactory nerve consists of two well-defined dorsal and

ventral bundles and each projects to the dorsal and ventral aspect of the homolateral bulb. Each olfactory nerve projects also to the contralateral bulb and piriform cortex. Only a small dorsal area of the hippocampus receives olfactory projections.

The existence of a commissural system interconnecting the cerebral cortex of the two hemispheres was established with electrophysiological technique.

It was found that the " general cortex " is divided into discrete areas which receive projections from different sensory modalities.

Acknowledgment

The project of studying the functional organization of the submammalian cortex on a comparative basis was suggested to us by Dr. George Bishop. His generous help and advice in all phases of this and the following three works is gratefully acknowledged. I am deeply indebted to Dr. Rita Levi-Montalcini for her cooperation and aid in the neuro-histological aspects of this investigation and for her constructive criticism and forebearing help in the preparation of these manuscripts.

REFERENCES

1. Adrian, E. D. Sensory discrimination with some recent evidence from the olfactory organ, *Brit. med. Bull.*, *6*: 330-332, 1950.
2. Allison, A. C. The morphology of the olfactory system in the vertebrates. *Biol. Rev.*, *28*: 195-244, 1953.
3. Bagley, C. and Richter, C. D. Electrically excitable region of the forebrain of the alligator. *Arch. Neurol. Psychiat., Chicago*, *11*: 257-263, 1924.
4. Bagley, C. and Langworthy, O. R. The forebrain and midbrain of the alligator with experimental transections of the brain stem. *Arch. Neurol. Psychiat., Chicago*, *16*: 154-166, 1926.
5. Beccari, N. *Neurologia comparata.* Firenze, Sansoni, 777 pp., 1943.
6. Bremer, F., Dow, R. and Moruzzi, G. Physiological analysis of the general cortex in reptiles and birds, *J Neurophysiol.*, *2*: 473-487, 1939.
7. Cajal, S. R. *Histologie du systeme nerveux de l'homme et des vertebres.* Madrid, Consejo superior de investigaciones cientificas, Instituto Ramon y Cajal, vol. I, 986 pp.; vol. II, 993 pp., 1957.
8. Crosby, E. C. The forebrain of Alligator mississippiensis. *J. comp. Neurol.*, *27*: 325-402, 1917.
9. Crosby, E. C. and Humphrey, T. The nuclear configuration of the olfactory and the accessory olfactory formations and the nucleus olfactorius anterior of certain reptiles, birds, and mammals. *J. comp. Neurol.*, *71*: 121-213, 1939.
10. Gamble, H. J. An experimental study of the secondary olfactory connexions in Testudo graeca. *J. Anat., Lond.*, *90*: 115-129, 1956.
11. Goldby, F. An experimental investigation of the cerebral hemispheres of Lacerta viridis. *J. Anat., Lond.*, *71*: 332-355, 1937.
12. Goldby, F. and Gamble, H. J. The reptilian cerebral hemispheres. *Biol. Rev.*, *32*: 393-420, 1957.
13. Herrick, H. The morphology of the forebrain of amphibia and reptiles. *J. comp. Neurol.*, *20*: 413-547, 1910.
14. Herrick, H. *Neurological foundations of animal behaviour.* Henry Holt, 334 pp., 1924.

15. Herrick, H. The amphibian forebrain. Cerebral hemispheres and pallial primordia. *J. comp. Neurol.*, *58*: 737-759, 1933.
16. Herrick, H. *The brain of the tiger salamander.* University of Chicago Press., 408 pp., 1948.
17. Herrick, H. *The evolution of human nature.* University of Texas Press, 506 pp., 1956.
18. Johnston, J. B. *The nervous system of vertebrates.* Blakiston, 370 pp., 1906.
19. Johnston, J. B. The cell masses in the forebrain of the turtle Cistudo carolina. *J. comp. Neurol.*, *25*: 393-468, 1915.
20. Johnston, J. B. Evidence of a motor pallium in the forebrain of reptiles. *J. comp. Neurol.*, *26*: 475-479, 1916.
21. Johnston, J. B. Further contributions to the study of evolution of the forebrain. *J. comp. Neurol.*, *36*: 143-162, 1923.
22. Kappers, C. U., Huber, G. C. and Crosby, E. C. *The comparative anatomy of the nervous system of vertebrates.* Macmillan, 2 vol., 1945 pp., 1936.
23. Kappers, C. U. *Anatomie comparée du système nerveux.* Paris, Masson, 754 pp., 1947.
24. Le Gros Clark, W. E. The projection of the olfactory epithelium on the olfactory bulb of the rabbit. *J. Neurol.*, *14*: 1-10, 1951.
25. Le Gros Clark, W. E. Inquires into the anatomical basis of olfactory discrimination. *Proc. Roy. Soc.*, B, *146*: 299, 1957.
26. Papez, J. *Comparative Neurology.* New York, Crowell, 518 pp., 1929.
27. Ramon, P. Neuvo estudio del encefalo de los reptiles. *Trab. Lab. Invest. biol. Univ. Madr.*, *15*: 83-99, 1917.
28. Ramon, P. Nuevo estudio del encefalo de los reptiles. *Trab. Lab. Invest. biol. Univ. Madr.*, *16*: 309-333, 1918.
29. Tuge, H. and Yazaki, M. Experimental note on the presence of electrically excitable areas in the reptilian cerebral hemisphere; clemmys japonica. *Sci. Rep.*, Tohoku Univ., *9*: 79-85, 1934.

THE REPTILIAN FOREBRAIN

II. Electrical Activity in the Olfactory Bulb[1]

F. ORREGO

Washington University, Department of Zoology
Washington University, School of Medicine, Division of Neurology
St. Louis (Mo.), U. S. A.

Introduction

The structural organization of the olfactory bulb and the olfactory pathways in the turtle were described in a previous paper (9). The present study is directed toward the analysis of the evoked and spontaneous activity in the olfactory bulb. This organ proved to be particularly favorable for electrophysiological analysis because of the simple geometrical distribution of its cellular layers which are displayed in an almost spherical configuration. The olfactory bulb is the first forebrain structure to have reached an advanced stage of organization maintained with only minor changes from lower to higher vertebrates. The olfactory bulb in higher vertebrates has the same basic structure as the olfactory bulb of reptiles. The history of the two main cellular components of the bulb, the granular cells and the mitral cells, is written down in the living vertebrates and can be traced step by step from lamprey to primate. This evolutionary process has been extensively investigated and was recently reviewed by Allison (3).

Finally, it should be pointed out that the structural organization of the olfactory bulb is very similar to the pattern of the cerebral cortex of higher forms, and it may well be considered as a simplified version of the latter. A functional analysis of the bulb is, therefore, of interest for the more general problem of functional organization in the cerebral cortical structures.

[1] This work has been supported by a grant from the Public Health Service, Number B-1602.

Methods

The materials and methods were described in the previous paper (9).

Results

1. *Characteristics of the potential evoked in the olfactory bulb by electrical stimulation of the olfactory nerve.* — A single shock applied to the olfactory nerve evokes (monopolar recording, surface to ventricle: Fig. 1): a relatively small potential which is generally

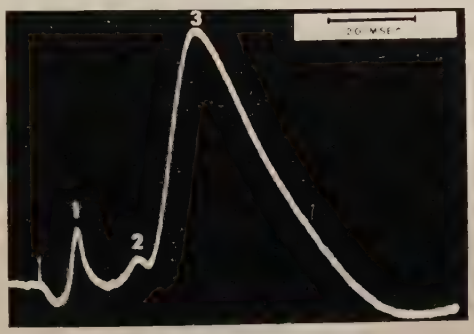


Fig. 1. – *Electrical stimulation of the olfactory nerve.*

Monopolar recording bulb surface to indifferent electrode in the ventricle. Upward deflection indicates surface-negativity.

diphasic with rather sharp ascending and descending phases. This potential is followed by a small slow surface-negative wave. In the descending phase or peak of this wave a third surfacenegative wave of high amplitude starts; it has a duration of 50 to 60 m/sec. A fourth slow wave of variable amplitude follows the third wave with about 100 milliseconds delay. This wave, however, is not always present.

2. *Sites of origin of the different components of the evoked potential in the olfactory bulb.* — Fig. 2 shows a mapping of the response in the olfactory bulb to olfactory nerve stimulation. The first wave is recorded clearly only in the anterior pole of the bulb where the olfactory nerve fibres are more densely packed, and its properties can be studied more accurately by leading from the olfactory nerve. The latency of the first wave increases in direct proportion with the distance between the stimulating and recording electrode in the olfactory nerve; the latency of the third wave instead (in this case recorded at a distance) remains constant (Fig. 3). This fact, as well as the diphasic or sometimes triphasic (positive-negative-positive) character of this first wave, strongly suggests that its origin is in

the olfactory nerve fibres themselves. Although the duration of this potential is rather long for an axon potential (10 msec or more), it must be remembered that we are dealing with exceptionally thin fibres (9). Potentials with similar duration were found by Gasser in the olfactory nerve of the pike (7). The second small negative wave is recorded all over the bulb; however, in the caudal part of the bulb it is less prominent because of the sharp ascending phase

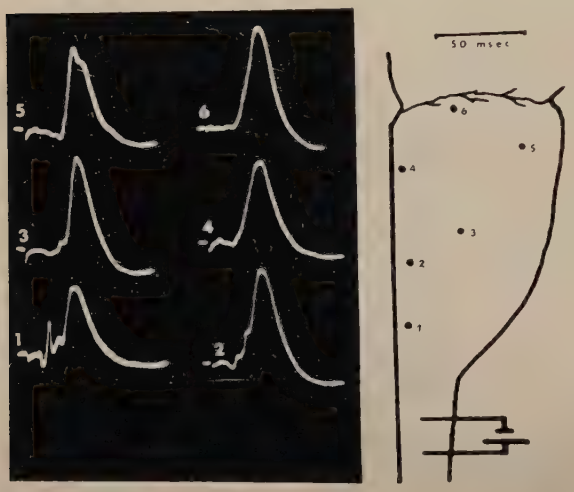


Fig. 2. – *Responses in different areas of the olfactory bulb to olfactory nerve stimulation.*

Monopolar recording surface to indifferent electrode. Upward deflection indicates surface-negativity.

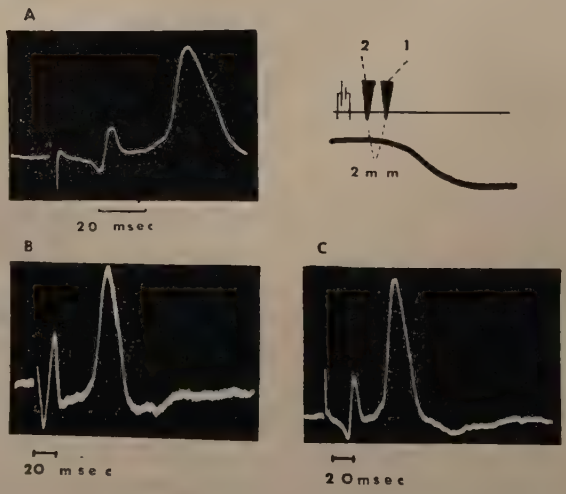


Fig. 3. – *Response evoked by electrical stimulation of the olfactory nerve recorded at different loci in the same nerve.*

The distance between recording electrode 2 and the cathode of the stimulating electrode is 2 mm, same distance beteen electrodes 2 and 1. Monopolar recording from surface to indifferent electrode. Fig. *B* recorded from electrode 2 to indifferent. Fig. *C* recorded from electrode to indifferent. Fig. *A* is the same as *C* but at a faster speed of the beam. Upward deflection indicates surface-negativity.

and large size of the third wave (records 4, 5, and 6 in Fig. 2 were taken at half the amplification of 1, 2, and 3). After a response of the olfactory bulb to nerve stimulation, there is a long period of depression to a second shock (Fig. 4). During this depression, the first potential, already described, and the second slow wave are not

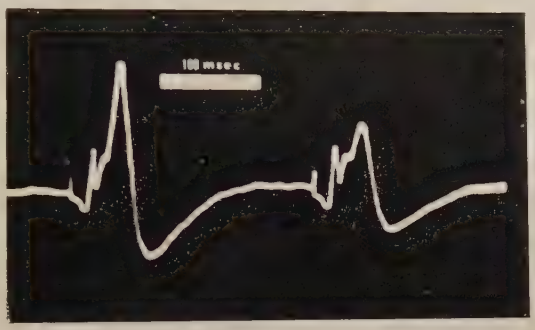


Fig. 4. *Response evoked in the olfactory bulb by two successive shocks delivered to the nerve.*

The first two components of the response to the second stimulus are not affected by the previous response while the third component is strongly depressed. Surface to ventricle recording. Upward deflection indicates surface negativity.

affected, but at short intervals between the shocks, the third wave is completely blocked. Therefore, the second wave and the third wave seem to arise from two independent processes. The antidromic response of the mitral cells evoked by stimulation of the olfactory

Fig. 5. – *Top record: antidromic response in the olfactory bulb to stimulation of the lateral olfactory tract.*

Middle record response in the olfactory bulb to stimulation of the olfactory nerve. The two presynaptic components of the response are marked with numbers. Lower record: orthodromic response preceded by antidromic response to stimulation of the olfactory tract. The first two components are not affected by the previous antidromic stimulation; the third is almost completely depressed. Monopolar recording surface to in different electrode. Upward deflection indicates surface-negativity.

tract completely blocked the third wave to subsequent orthodromic stimulation without affecting the first or second wave (Fig. 5). When using fractional recording with the leads at different depths in the bulb, the second wave shows only in the more superficial leads with

a reversal of its polarity rather close to the surface. These results suggest that the second wave represents activity in the fine intraglomerular presynaptic fibres and/or synaptic potentials at the tips of the apical dendrites of the mitral cells.

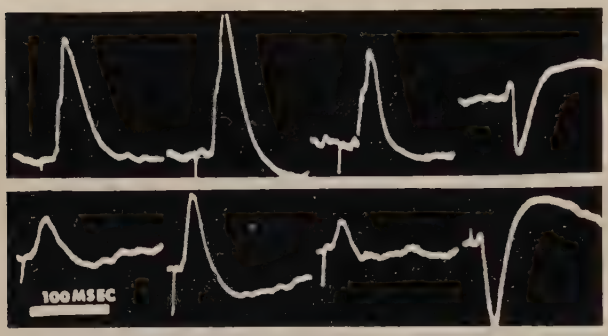


Fig. 6. – *Upper row: response in the olfactory bulb to electrical stimulation of the olfactory nerve.*

Monopolar recording with an 8 μ needle. From left to right the depth of recording is increased. Lower row: response in the olfactory bulb to olfactory tract stimulation recorded at the same depths as in the upper row. Upward deflection indicates negativity at the point of recording.

The third wave represents the main component of this complex potential as can be seen in Fig. 1. Its amplitude may be several

Fig. 7. – *Transverse section through the olfactory bulb (toluidin stain).*

The arrow indicates the point of iron deposit by the electrode placed in the position where the potential reverses in Fig. 6.

millivolts, even with extracellular recording. Fig. 6 shows the response to orthodromic and antidromic stimulation at different depths. Both types of response reverse at approximately the same depth which is marked by iron deposit in Fig. 7. The experiment illustrated

in Fig. 8 shows similar results, but in this case instead of recording at different depths against an indifferent electrode, we used the technique of fractional recording. In this case, the exact depth of each lead was known. In the experiment with monopolar recording, the third potential reverses to a negative-positive potential at the level of the cell bodies and axons of the mitral cells regardless of whether the stimulation is orthodromic or antidromic. The maximum negativity is not at the surface, but at a deeper level. This point is even more clearly demonstrated by fractional recording where it can be seen again that the maximal negativity is at the level of the basal dendrites and cell bodies of the mitral cells. These results strongly suggest that this third large component of the bulb potential is originated mainly in the depolarization of the basal dendrites and/or cell bodies of the mitral cells. A tentative interpretation of these findings will be given in the discussion.

In Fig. 8 (lead 1-5), as well as in Fig. 5, the response to orthodromic stimulation shows clearly the small wave that preceeds the

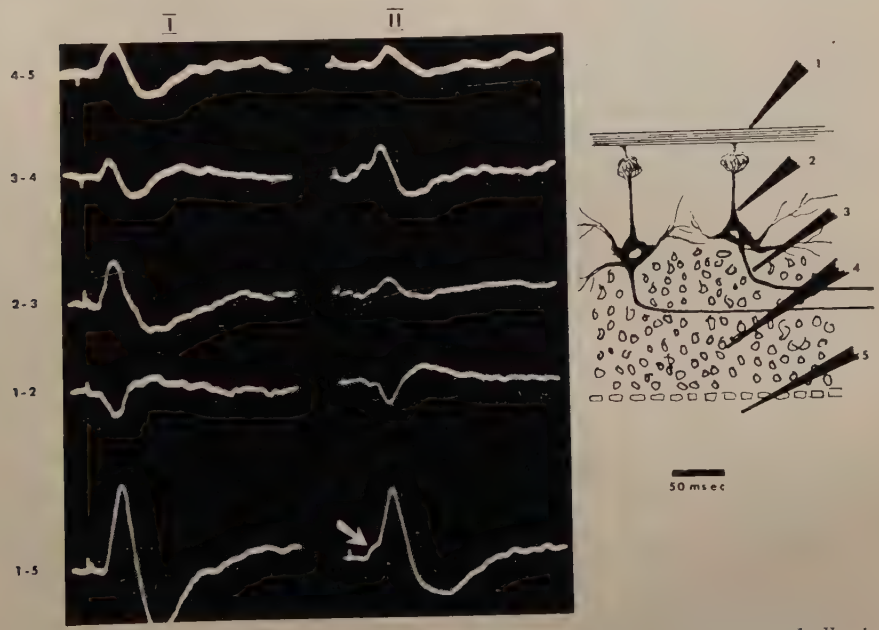


Fig. 8. – *Fractional recording of the response of the olfactory bulb to orthodromic and antidromic stimulation.*

The tip of each lead is placed at the points indicated in the drawing. Column I: responses to antidromic stimulation. Column II: responses to orthodromic stimulation. Upward deflection indicates a positive potential.

third wave under discussion. This small wave (2 wave) is completely absent in the case of antidromic stimulation, strengthening the hypothesis that this might represent glomerular and synaptic activity.

Fig. 9 *A* represents the response recorded at the surface of the bulb evoked by olfactory nerve stimulation. In *B* and *C* recording was done just above the level at which the evoked response reverses in polarity. A small needle with a tip no larger than 8 μ in diameter

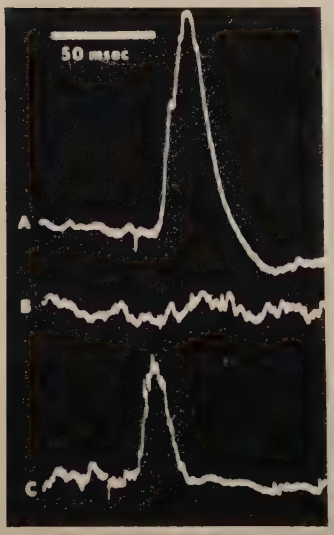


Fig. 9. – *A*: response at surface of the bulb to olfactory nerve stimulation. *B*: spontaneous activity recorded close to the internal granular layer. *C*: response evoked by stimulation of the olfactory nerve recorded at the same locus as record *B*. Same amplification used in all three records. Notice absence of spontaneous activity in the surface recording.

was used. The spontaneous activity is illustrated in record *B*. This activity does not show in surface recording and it grows in size as the recording electrode is pushed deeper into the bulb. In record *C*, the spontaneous activity clearly precedes the shock and continues in the ascending phase and part of the descending phase of the slow wave evoked by olfactory nerve stimulation. After the slow wave, there is a " silent period " and then a rebirth that builds the fourth wave mentioned above. The place of recording, as well as the events that will be analyzed in a following paper, very strongly suggest that this activity originates in the granular cells. The granular cells that normally show asynchronic spontaneous activity must then be activated through the axons of the mitral cells to a rather synchronic discharge that contributes to the third wave, chiefly the response of mitral cells. This synchronous discharge is followed by a silent period and then bin a renewed discharge.

3. *The excitability cycle following response in the olfactory bulb to a single shock in the olfactory nerve.* — After the response in the olfactory bulb to a single shock delivered to the olfactory nerve, there is a long-lasting depression to a second shock at the same site. If the interval between the two shocks is shorter than 10 msec, the response is larger than the response to a single shock; however, it never actually doubles in size. This might be due to temporo-spatial summation at the glomerular synapsis; the first shock might fire some of the mitral cells and evoke a subliminar fringe in other glomeruli which are brought to threshold level by the second shock. After this short period of facilitation, there is a period of complete depression lasting 500 msec or longer with maximal stimulation. The duration depends also on the general condition of the preparation. As was mentioned earlier, only the third and fourth wave of the responses of the olfactory bulb are depressed during this period. This phase of complete depression is followed by a period of partial depression lasting as long as 15 sec. The excitability cycle in the piriform cortex following olfactory nerve stimulation almost exactly parallels, as might be expected, the olfactory bulb cycle.

4. *Response in the piriform cortex to stimulation of the olfactory bulb at different depths.* — In order to determine the locus of this depression, the stimulation was applied to the olfactory nerve and the recording was made in the piriform cortex. First, we will give a brief description of the technique used.

Stimulation of the surface of the bulb with a small bipolar electrode (two 20 μ wires cemented together and filed to a sharp tip), gives origin to a response in the posterior piriform cortex which is similar to the response obtained by olfactory nerve stimulation. The latency of this response is about 25 msec. If the stimulating electrode is pushed a small fraction of a millimeter into the bulb, the latency of the response is decreased by 8-10 msec without changing its general characteristics. The structure responsible for this change in latency is represented in Fig. 10. The two tips of the stimulating electrodes are separated by a distance of 50 to 60 μ in depth. The electrode is placed in such a way that the pole closest to the surface is at the glomerular level or superficial to it, and the deep pole is in the external plexiform layer just below the glomerular level. In the record from the piriform cortex, as illustrated in Fig. 10, the response with shorter latency was evoked by making the deep

pole the cathode; the superimposed record with longer latency was obtained by using the superficial pole as a cathode. Apparently then, this difference in latency is caused by a long delay necessary for transmission within the glomeruli including not only the synaptic mechanism itself, but also transmission through the fine intraglomerular fibres.

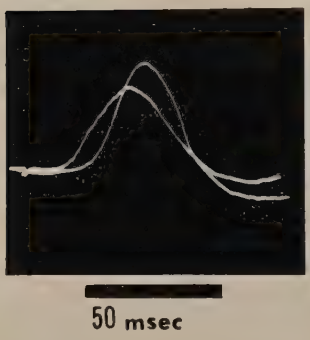


Fig. 10. – *To the left, two superimposed records of the response in the piriform cortex to olfactory bulb stimulation.*

Response with shorter latency; cathode: deep stimulating electrode. Response with longer latency, cathode: superficial electrode. The distance between the two tips of the stimulating electrode is 50 μ.

The results of the following experiments are also in agreement with this interpretation. The diagram in Fig. 11 shows the position of the stimulating electrodes. Electrode *A* is placed in the olfactory nerve; *B* in the surface of the bulb just above the glomeruli; and *C* just below the glomeruli. The recording electrode is in the piriform cortex. The records in the top row are control responses to single shocks applied in the three different locations. The difference in size between the responses is not significant and varies from one experiment to another according to the topographical relations between the point of stimulation and the point of recording. The records in the bottom row are responses to single shocks in the same locations as above after tetanic stimulation to the nerve (30 shocks per second for 20 seconds). These records show clearly that there is a strong potentiation of the response to single shocks applied to the nerve and to the preglomerular fibres, but no change in the response to post-glomerular stimulation. It is generally agreed that the post-tetanic effect is due to change in the pre-synaptic elements (10). The tetanus to electrode *A* affects the post-tetanic

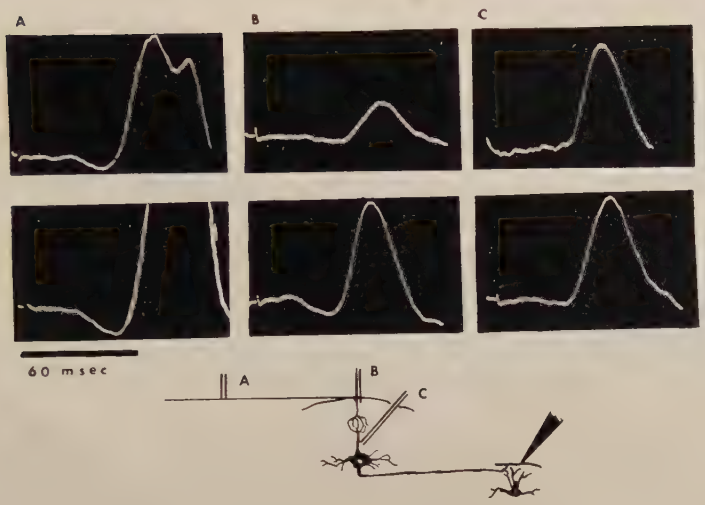


Fig. 11. – Upper row. *A*: response in the piriform cortex to stimulation in the olfactory nerve. *B*: response in the piriform cortex to stimulation in the surface of the bulb. *C*: response in the piriform cortex to stimulation in a point immediately below the glomerulus. Lower row: response to stimulation in the same loci after tetanic stimulation through electrode *A*. Upward deflection indicates surface-negativity.

response to single shocks in electrode *A* and *B* because these two electrodes are in a pre-synaptic position, while the response from stimulation at *C* is not affected because it is in a post-synaptic position [1].

5. *Locus of depression following a response to a single shock in the olfactory nerve.* — In the experiment illustrated in Fig. 12, the

---

[1] Some aspects of the post-tetanic effect in the glomerular synapsis ask for a comment. In some synaptic systems in mammals and also in the turtle, the predominant post-tetanic effect is one of facilitation; in the glomerulus there is a strong and long-lasting depression not followed by facilitation. Only the repetition of the tetanus for long periods of time and/or the deterioration of the preparation change the post-tetanic depression into post-tetanic potentiation. Only exceptionally a preparation in good condition shows post-tetanic potentiation after the first few trials. Regardless of whether the effect of the tetanus is depression or potentiation, the experiment described above always results in the same cross effect between electrodes *A* and *B* and no change in the response to stimulation in *C*. The frequencies that are successful in evoking post-tetanic effect are between 20 and 40 cycles per second for durations of 20-40 sec.

position of the stimulating electrodes 1 and 2 was similar to that of electrodes *B* and *C* in the previous experiments. This position was checked by the difference in latency as shown in row *A*, by the post-tetanic effect and histological control. A third stimulating electrode was placed at the origin of the lateral olfactory tract in the margin between the olfactory bulb and thehemisphere. The recording electrode was placed in the piriform cortex. Row *B* shows records of the response to two shocks delivered through the different stimulating electrodes at a similar interval. There is no response to the second shock of the pre-glomerular stimulation. There is a response with a certain degree of depression to the second shock in post-glomerular position and there is facilitation to the second shock delivered to the axon of the mitral cells. Therefore, one locus of depression must be in the nerve fibres or in the glomeruli themselves. It can be demonstrated that the nerve fibre is not responsible for this depression by delivering two shocks to the olfactory nerve and recording in the nerve itself. This experiment shows that after a short period of depression not much longer than the duration of the action potential itself, there is a slight degree of facilitation of the response to the second shock.

Another proof that the depression following the response in the olfactory bulb is not only due to a post-activity depression of the mitral cells is the fact that even a sub-threshold conditioning stimulus can strongly depress the response to subsequent supra-threshold stimulation (Fig. 13). The records show the response to combinations of two shocks delivered through two different electrodes at the same intensities as the control but at increasing intervals; the first shock is always the weakest. At short intervals, there is a facilitation of the response to the supra-threshold stimulation by the conditioning sub-threshold stimulus. This result may be explained by the spatio-temporal summation which is likely to take place in the glomerulus. We have in fact a high degree of convergence of nerve fibres (more than 15,000 olfactory nerve fibres converging in each glomerulus) (3). This short facilitation is followed by a long-lasting depression as illustrated in Fig. 13. Two alternative hypotheses could explain this last phenomenon: *a*) the existence of inhibitory fibres of low threshold in the olfactory nerve *b*) a non-recordable response to the sub-threshold stimulation which will result in the depolarization of the intraglomerular fibres and partial activation of the synaptic field in the glomerulus without resulting in firing of the mitral cells.

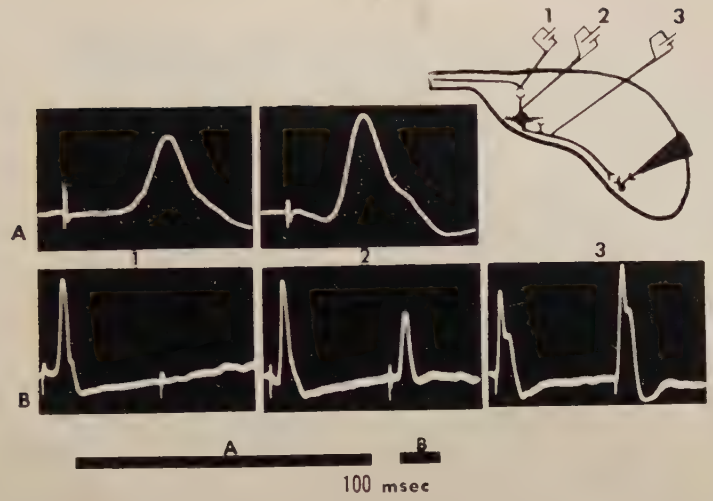


Fig. 12. – Row *A*, response in the piriform cortex to stimulation above and immediately below the glomerulus. Row *B*, response in the piriform cortex to two shocks around 250 msec apart delivered to point 1, point 2, and point 3. Point 3, in the axons of the mitral cells in the boundary between bulb and cortex. Upward deflection indicates surface-negativity.

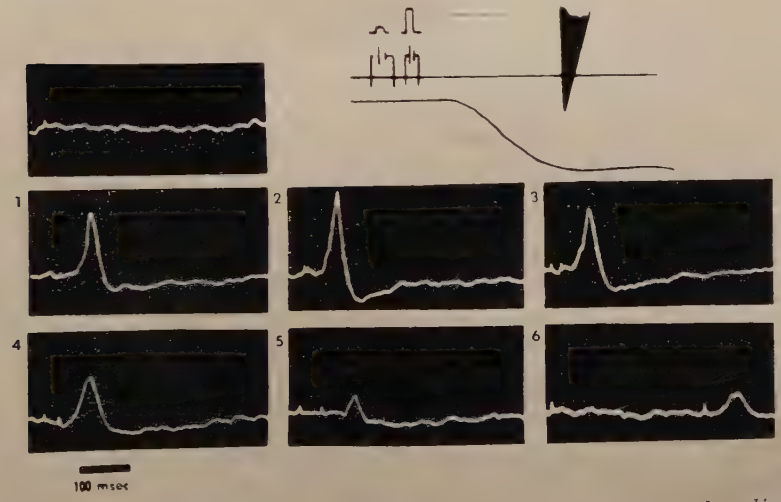


Fig. 13. – *Upper left record, subthreshold stimulation in the olfactory nerve.*

Record below: response evoked by threshold stimulation of the olfactory nerve recorded in the olfactory bulb. In the following records the subthreshold and threshold stimuli are delivered at increasing delays. The weak shock always precedes the strong shock. Upward deflection indicates surface-ngativity.

The early facilitation, as described above, makes the first hypothesis unlikely [1].

Fig. 12 suggests that another locus of depression may be present. As shown in the diagram, electrode 2 is located in the layer of the apical dendrites of the mitral cells, and electrode 3 is situated in the layer of axons of the same cells. The cell body of the mitral cells and the recurrent branch of the axon is interposed between the two stimulating electrodes. The recurrent axon was investigated by Cajal in mammals (5). He found that it branched in the internal granular layer. Our studies in turtles agree with these findings. Cajal also states that the short axons of the internal granular cells end on the basal dendrites of the mitral cells, thus completing a recurrent circuit. We were unable to see the actual termination of the recurrent axon in our Golgi material, but the available evidence suggests a connection from it through the granular cells with mitral cells other than the cell of origin. The record in Fig. 12 shows that facilitation instead of depression occurs when the stimulus is applied in position 3, that is, to the axons of the mitral cells. The facilitation is not dependent in this case upon the point of stimulation, but upon the characteristics of the synaptic field in the piriform cortex. These synaptic fields will be analyzed in detail in a following paper. Since no facilitation, but slight depression follows when the stimulation is applied proximal to the point of emission of the recurrent axon, this result suggests that the circuit through this axon is responsible for the depression. A support to this hypothesis is presented in the next paper.

The following experiment rules out the possibility that we are dealing with two different pathways, one giving origin to post-activity facilitation, and the other to post-activity depression. In this experiment (Fig. 14), the stimulating electrodes are placed in the same position as above: one in the external plexiform layer and the other at the origin of the olfactory tract. The recording electrodes are placed in the piriform cortex. Records *A* and *B* show a response to two shocks delivered through electrode 1 and electrode 2 respec-

[1] The two shocks are delivered through different electrodes in order to avoid any possible influence of polarization of the electrode after the first shock. The stimulating electrodes are only 20 μ in diameter and consist of stainless steel wire that is easily polarizable. The relative positions of the two stimulating electrodes are most important because the intensity of the stimulus is very low and allows very little spread of current through the nerve.

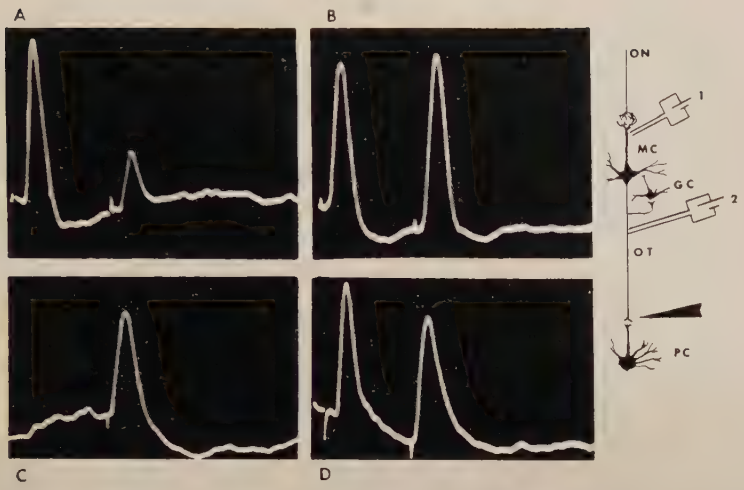


Fig. 14. – *A*: response to two shocks through electrode 1 in the external plexiform layer. *B*: response to two shocks through electrode 2 at the origin of the olfactory tract. Recording electrode in the piriform cortex. *C*: response to single shock through electrode 2. *D*: response in the piriform cortex to a single shock through electrode 2 preceded by a single shock to electrode 1. Upward deflection indicates surface-negativity in the piriform cortex. ON, olfactory nerve; MC, mitral cell; GC, granular cell; OT, olfactory tract; PC, piriform cortex.

tively. Record *C* is a control to a single shock applied to electrode 2 and record *B* shows a response to a single shock of the same strength applied in the same location when preceded by a shock through electrode 1. The response to a shock delivered to an axon is clearly potentiated by the preceding dendritic stimulation; thus proving that we are not dealing with a different pathway.

Fig. 15 shows a graphic representation of the excitability cycle following the response evoked by stimulating the olfactory nerve (or surface of the bulb), the external plexiform layer, and the olfactory tract. The recording electrode is in the olfactory bulb; therefore, the response to olfactory tract stimulation is an antidromic response. Early facilitation is absent in the excitability cycle following stimulation of the external plexiform layer. There is a late facilitation which is never present in the excitability cycle following olfactory nerve stimulation. These different excitability cycles can be obtained by changing the stimulation from the glomerular level to a point slightly below it; the difference in depth being not necessarily greater than 50 μ. The absence of early facilitation to deep stimulation

might be explained by the fact that the stimulus is delivered to a point beyond the convergence of olfactory nerve terminals in the glomeruli that structurally favor spatial summation. This fact, in addition to the post-tetanic effect, the histological control, and the

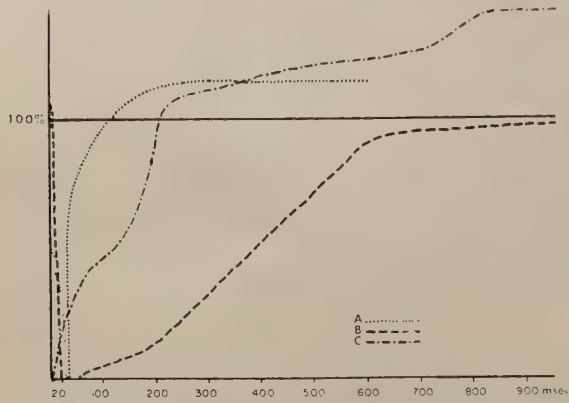


Fig. 15. – *Graphic of the excitability cycle following the response of the olfactory bulb to.*

A: local stimulation in the external plexiform layer immediately below the glomerulus; *B*: stimulation in the olfactory nerve, and *C*: stimulation in the olfactory tract (antidromic response). Plotting on different lines represents areas circumscribed by potentials to second shocks at different delays. Compare with reference line at 100% representing response to simple shock. With very short delay between two stimuli the responses were superimposed. In this case, the total area was measured and the area of the response to a simple shock subtracted from this.

similarity between post-glomerular and antidromic cycles favors the inference of a direct electrical excitation of the apical dendrites, especially since there are no synaptic contacts on the dendrites in the region stimulated.

6. *Response in the olfactory bulb pathway to repetitive stimulation.* — As one could expect from the characteristics described of the excitability cycle of the olfactory bulb following a single shock applied to the olfactory nerve, the response to repetitive stimulation is strongly decremental at frequencies as low as one per second. Frequencies of stimulation from 6 to 9 per second give origin to a peculiar and constant pattern of response; after a first decremental phase, the responses increase in size with each following shock until finally an alternating pattern is established, and this pattern then continues for the duration of the stimulation (Fig. 16). The mechanism of this alternating response might involve the effect of the inhibitory feedback represented by the circuit " recurrent axon → granular cell → basal dendrite of the mitral cells ".

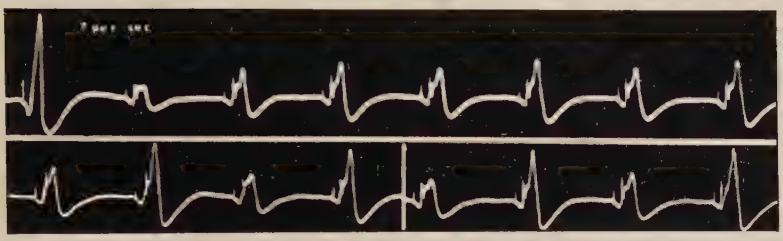


Fig. 16. – *Response in the olfactory bulb to repetitive stimulation of the olfactory nerve. Upward deflection indicates surface-negativity.*

If the response to repetitive stimulation is recorded in the piriform cortex, the pattern is dependent upon the point of application of the stimulus, *i. e.*, if the stimulus is pre-glomerular a strongly decremental response is obtained; if post-glomerular, the response is slightly decremental, and if axonic, an incremental response is evoked.

Discussion

This paper deals with the sequence of events following electrical stimulation of the olfactory nerve in turtles.

The turtle has a well-defined olfactory nerve containing compact bundles of fibres, which make possible its synchronous activation by electrical shock. This type of stimulation would not be feasible in most mammals since the olfactory fibres run separately instead of being collected in well-defined nerve bundles. The synchronous activation of many neurons offers the possibility for precise measurement of the time sequence of the electrical events. An attempt was made to correlate each component of the evoked activity with its locus of origin in individual nerve structures.

Synaptic conduction through the glomerulus is extremely slow requiring 8 to 10 msec. This might be due to the intrinsic synaptic process or to the slow conduction in the intraglomerular fibres which are extremely thin. The existence of a slow pre-synaptic potential with characteristics different from those of the action potential of the axons themselves was well established.

The site of origin of the different components of the complex evoked activity was determined by combining different techniques of stimulation and recording.

Records from different depths, following either orthodromic or antidromic stimulation, are identical in showing the maximum ne-

gativity of the most prominent component of the response at or immediately above the mitral cell bodies, and the maximum rate of change, — to +, just below this, in the internal plexiform layer. Here, this potential reverses with respect to a distant reference lead, locating a boundary between active and inactive tissue. At this level, the record also becomes diphasic, showing a brief negative spike preceding the larger positive wave. These findings relate this brief spike to the axon hillock and the much larger negativity to the dendritic pole of the cell body, with possible involvement of at least the proximal portions of its dendrites. No diphasicity is present in the dendritic region such as would indicate conduction upwards toward dendritic terminals. The deep positivity is then a reflection of the more shallow negativity in a dipolar field around an active neuron in a conducting medium. The initial spike negativity appears to be the immediate precursor, at the axonal pole of the cell, of the much larger potential occupying the dendritic pole.

In the analysis reported above, no attempt was made to pursue a detailed study of the electrical field as was performed by other authors in other nerve structures. We believe, however, that the negative-positive sequence in the deeply recorded potential, evoked by orthodromic or antidromic stimulation, is an indirect, but strong argument in favor of the hypothesis proposed above.

These results justify the following tentative interpretation of the sequence of events following stimulation of the olfactory nerve. The incoming impulse depolarizes the terminals of the apical dendrite in the glomerulus; this synaptic activity, in turn, depolarizes electrotonically the cell body, resulting in a spike response of the axon hillock and axon. The spike potential, much greater than the electrotonic depolarization that initiates it, is then capable of exciting the cell bodies and dendrites. Such an hypothesis was first suggested by Coombs, Curtis, and Eccles in their investigation of the activity of spinal motor neurons activated through the reflex pathway (6). The over all surface-negativity, when recorded from surface to ventricle, is the algebraic summation of the negatively directed dendritic pole activity as sink, and the positivity of the axonal pole as source. The inequality, with resulting net surface-negativity, is presumably due to the asymmetry of the neurons and of the field about them. This asymmetry may include a decremental spread of depolarization along the dendrites toward the glomeruli, electrotonic or graded in character.

The results discussed above concern the evoked activity in the mitral cells. The following discussion will be concerned with the activity in the granular cells. The maximum spontaneous activity in the olfactory bulb is recorded with electrodes placed deep in the internal granular layer. As shown in Fig. 9, this activity of the granular cells goes through a "silent period" when a response is evoked after electrical stimulation of the olfactory nerve. This fact strongly suggests that the granular cells are driven into activity every time impulses travel through the mitral cell pathway. The granular cells do not receive direct connections from the olfactory nerve, but they are indirectly connected with the nerve by the recurrent axon of the mitral cells. In mammals the recurrent axons of the mitral cells end on the granular cells (5). Although in the turtle we were unable to see the actual endings of the recurrent axon, we saw this axon branching in the direction of the granular cells shortly after its origin. Some of the granular cells, in turn, send their axons to the basal dendrites of the mitral cells; thus closing a kind of "recurrent circuit". Cajal (5) and most authors after him, have interpreted these morphological findings as indicative of a mechanism for spreading and reinforcing of activity to neighboring units as well as refiring of the same system after a simple afferent volley. This interpretation does not seem to apply to our results in the turtle. The activity in the above mentioned recurrent system blocks the pathway to impulses coming by way of the glomerulus. As described in previous paper (9), the "recurrent circuit" apparently ends in mitral cells other than the cell of origin. If any speculation can be advanced on its functional meaning, it will be that rather than diffusing the activity, it will tend to restrict it to a particular group of cells and inhibit the other mitral cells. Adrian (2) found that different mitral cells are sensitive to different odors. The above mentioned mechanism may play a role in such a differential sensitivity. Many other pathways end in the granular cells (see following paper). They all have the same inhibitory effect upon the mitral cells.

The characteristics of the excitability cycle in the pathway from nerve to cortex deserve comment. As mentioned in the results, there are two main loci of inhibition in this pathway: one is in the glomeruli and the other in the basal dendrites of the mitral cells. At the glomerulus, the post-activity depression to maximal stimulation of the nerve lasts for an extremely long time. However, even

a weak shock, unable to evoke any "recordable response", blocks a response of the bulb to a following strong shock applied to the nerve. The weak shock may result in activity in a large number of synapses in the glomerulus. Such activation may, however, be subliminal and no depolarization of the cell body and dendrites of the mitral cells will follow [1]. The stronger subsequent shock will find these synapses in a "depressed state", and the amount of spatial summation necessary to fire the mitral cell will not be reached. The unusually high amount of convergence taking place at the glomerulus strongly suggests that the normal way of activation at these particular synapses needs a high degree of spatial summation. The second locus of depression at the basal dendrites of the mitral cells was already discussed. In this case, the depression is due to true inhibition and not to post-activity depression. The stimulation of other pathways which also end in the granular cells blocks the response to olfactory nerve stimulation. In this case, the inhibition is not preceded by activity of the mitral cells or activity at the synaptic level.

Three types of responses were observed in the piriform cortex through repetitive stimulation at three different points of the olfactory pathway. The stimulation of the glomerulus, in a presynaptic locus, results in a strongly decremental response; post-synaptic stimulation immediately below the glomerulus results in a slightly decremental response, while stimulation of the olfactory tract beyond the origin of the recurrent axon evokes a recruiting response. Since the responding elements are the same in all three instances, it is shown that the response can shift from decremental to recruiting according to the place of stimulation in the same pathway, even when no synapse is interposed. In other words, a change from a decremental to a recruiting response by shifting the locus of stimulation does not necessarily imply a change of the stimulated pathway or of the responding elements.

Summary

The evoked response in the olfactory bulb to electrical stimulation of the olfactory nerve was analyzed. The site of origin of the different components of the complex evoked potential was investi-

[1] The presynaptic response is very small even to a maximal stimulation; so it does not show with weak stimulation.

gated by the technique of differential fractional recording, and the histological localization of the recording leads by ferrocyanide staining. The evoked activity consists of four main components. The first is the action potential of the olfactory nerve fibres; the second is the presynaptic activity of the fine intraglomerular fibres; the third is the response of the dendrites and/or the cell bodies of the mitral cells; the fourth is the activity of the granular cells. The spontaneous activity in the olfactory bulb originates in the granular cells.

A very long lasting depression (10-15 sec) was observed in the piriform cortex following a response to a maximal stimulation of the olfactory nerve. Two loci in the pathway seem to be responsible for it. The first is a post-activity depression in the glomeruli; the second is the inhibitory feedback through the recurrent axon of the mitral cells and granular cells. Repetitive stimulation of the olfactory bulb results in three different patterns of activity; a strongly decremental response to presynaptic glomerular stimulation, a slightly decremental response to postglomerular stimulation, and an incremental response to stimulation of the axon of the mitral cells beyond the origin of the recurrent axon. Thus, a response can change from decremental to recruiting by shifting the locus of stimulation along a pathway, even if no synapse is interposed.

REFERENCES

1. Adrian, E. D. Sensory discrimination. *Brit. med. Bull.*, *6*: 330-332, 1950.
2. Adrian, E. D. The action of the mammalian olfactory organ. *J. Laryng. Otol.*, *70*: 1-14, 1956.
3. Allison, A. C. The morphology of the olfactory system in the vertebrates. *Biol. Rev.*, *28*: 195-244, 1953.
4. Beidler, L. M. and Tucker, D. Response of nasal epithelium to odor stimulation. *Science*, *122*: 76, 1955.
5. Cajal, S. R. *Studies on the cerebral cortex*. Chicago, Year Book Publishers, 179 pp., 1955.
6. Coombs, J. S., Curtis, D. R. and Eccles, G. C. The interpretation of spike potentials of motoneurons. *J. Physiol.*, *139*: 198-231, 1957.
7. Gasser, H. S. Olfactory nerve fibres. *J. gen. Physiol.*, *39*: 473-496, 1955.
8. Mozell, M. and Pfaffmann, C. The afferent neural processes in odor perception. *Ann. N. Y. Acad. Sci.*, *58*: 96-108, 1954.
9. Orrego, F. The reptilian forebrain. I. The olfactory pathways and cortical areas in the turtle. *Arch. Ital. Biol.*, *99*, 425-445, 1961.
10. Wall, P. D. and Johnson, A. R. Changes associated with post-tetanic potentiation of a monosynaptic reflex. *J. Neurophysiol.*, *21*: 148-158, 1958.

ANALYSES

Handbook of Physiology, Section I. Neurophysiology. Vol. II. American Physiological Society, Washington, D. C., 1960, VI, 781-1439 pp.

The second volume of the neurophysiology section of the new Handbook of Physiology covers most of what is called "regional neurophysiology". The material has been grouped under two main headings, (I) motor, or rather effector, mechanisms; and (II) central regulatory mechanisms, each section being introduced by such established authorities as Professor Denny-Brown and Professor Bremer, respectively.

The motor control in the somatic sphere is dealt with in several separate chapters devoted to sensori-motor integration, pyramidal and extrapyramidal activities, spinal mechanisms, posture and locomotion, eye movements. Central and peripheral autonomic mechanisms (a classification which seems to follow Langley's old conception of the autonomic system as a purely motor outflow) are also discussed, with special accounts of neural regulation of different visceral functions (pituitary secretion, respiration, circulation, digestion, etc.). A separate discussion of such complex phenomena, as feeding, drinking, and reproduction, is also included, although these functions can hardly be considered merely effector in nature. It is not clear, however, why four chapters on central and peripheral autonomic mechanisms, on the central control of pituitary secretion and on neurosecretion were inserted in this section between those devoted to spinal reflexes and to posture and locomotion.

Under the heading of Central regulatory mechanisms, classical topics such as the cerebellum (which might have been discussed with the motor mechanisms) are found together with newer subjects, such as the brain stem reticular formation, the unspecific thalamic nuclei, and the several structures belonging to what is now called the limbic system. Emphasis on the ascending functions of the reticular formation and their relation to wakefulness and sleep (one of the most important neurophysiological contributions in recent years) comes however unexpected to the reader of an earlier chapter where the reticular formation is defined as a motor center, and arguments are raised against "the present vogue of brain mythology about consciousness and attention" (p. 913).

Detailed analysis of the single chapters would easily lead to trivial criticisms, irrelevant for a book of this kind, and the reviewer feels tempted to accept the editor's invitation to judge from the pages of this book "the status of *neurophysiology* just past the midmark of the twentieth century" (Vol. I, p. XI), or at least the success attained by both editors and authors in representing the spirit and the accomplishments of to-day's neurophysiology.

Clarity of thought and perspicuity are outstanding almost in every chapter, thus making the volume a faithful reflection of the empiricism of British and American tradition which has marked the growth of physiology in the first part of this century. Reference is made throughout to factual data, without undue recourse to generalization or excessive speculation, thus offering some contrast with the older treatises of German tradition.

A few deviations from this line are due to the editor's effort to include authors of different background and philosophy, an endeavor which has otherwise contributed to the high standing of the volume. This has resulted in occasional slips from the usual operational rigour of the language.

The volume here reviewed also offers an opportunity to judge how fruitful this new spirit and modern technology have been in terms of actual experimental results. Throughout these pages, one comes across a large part of the most important contributions which have marked the progress of modern neurophysiology. Indeed, in older handbooks, and even in those which have immediately preceded the present one, one could hardly find the mere concept, e. g., of postural regulation through the gamma loop, or of pituitary control through the hypophysial portal vessels; nor was the attempt to interconnect subcortical and cortical functions by way of the " diffuse " reticular and thalamic systems even conceived only twenty years ago.

These and other accomplishments are all faithfully and carefully expounded in the twenty seven chapters of the volume. Over five thousand references testify the authors' efforts in assembling such a huge body of scientific material and in balancing the proportion of old and newer contributions. They testify moreover how actively and fruitfully neurophysiological investigation has been pursued during the last decades. In almost every chapter the body of knowledge appears to have substantially grown in post-war years.

However, at the end of his reading the neurophysiologist is left with a mixed feeling of pride and perplexity. So many advances in techniques and in information on separate fields seem hard to weave together to yield a more coherent or, at least, a less confused view of the brain functions as a whole. Since a spirit of integration can scarcely be perceived in the book, the reader is left with the impression of having perused an invaluable sourcebook of information, a vast and wealthy repository of useful references, rather than a unified compendium of neurophysiological knowledge. Although the editors might have provided a considerably more co-ordinated work, one remains with the doubt that the sourcebook character of the Handbook may be substantially consonant to the nature and the aims of to-day's biology. Refinement of techniques and increasing emphasis on unitary phenomena have brought with them, as a necessary complement, fragmentation, deficient systematization, lack of far-reaching insight. While avoiding lamentations for a trend which seems necessary to the growth of his science, the neurophysiologist has to accept broader integration as a task to be deferred to some future generation of scientists.

A. Zanchetti

Gellhorn E. *Autonomic Imbalance and the Hypothalamus. Implications for physiology, medicine, psychology, and neuropsychiatry.* University of Minnesota Press, Minneapolis, 1957, xiv-300 pp., $ 8.50.

Both this monograph and the more recent one by Sager (see below) are intensely personal in nature. As a result, they exhibit the advantages and the disadvantages which always accompany such an approach. The two works complement each other in a unique way, however, and in combination provide a remarkable overview of tae diencephalon.

Gellhorn's monograph is a personal statement in the grand manner. Ranging over wide areas of the Western literature, but always heavily dependent upon his own investigations, Gellhorn surveys the role of the hypothalamus in many diverse autonomic functions. The monograph is not only " .... a study of the influence of reflexly and directly produced central autonomic imbalances on autonomic reflexes and, to use Cannon's terminology, on the sympathetic and para-sympathetic ' downward ' discharge. Fundamental as such work appears to be for physiological and

clinical problems, it needs an important complement, the investigation of the 'upward'—hypothalamic-cortical—discharge under the conditions of an altered hypothalamic autonomic balance." (p. 5). With this point of view as a starting point, and analyzing hypothalamic autonomic balance in terms of the relative strength of sympathetic and para-sympathetic discharge, Gellhorn goes on to discuss in a most stimulating manner the implications of his studies on hypothalamic mechanisms to a wide range of neurophysiological, psychological, and neuropsychiatric problems. The author has no doubt that his " .... program for neuro-psychiatric research and his practical suggestions will be rejected by some as speculation or dreams ". To such critics, he has two answers: " (1) facts as such are sterile and only ideas grown from well established facts are responsible for the advancement of science; (2) history has shown that not the realists but the dreamers have conquered the world. " (p. 281).

Both Gellhorn and Sager make a point of addressing their books to the physician in the hope that the information on diencephalic mechanisms will assist him in the care of patients. To this reviewer, however, it seems that they will be chiefly of interest to the serious student of the thalamus and hypothalamus, who will find much to ponder.

R. J. Gumnit

Sager O. *Diencefalul*. Editura Academiei Republicii Populare Romine. Bucaresti, 1960, 364 pp., Lei 34, 80. (In Romanian with summary and figure legends also in Russian and English).

This reviewer does not read Romanian, therefore the comments on *diencefalul* are based upon the 17 page English summary and the English translation of the legends of 208 figures.

Sager's book actually consists of two independent parts. The first half of the monographs is devoted to a survey of the phylogeny and anatomy of the specific and non-specific thalamic nuclei. Referring frequently to classical and to some forgotten papers in the early literature, Sager outlines the anatomy of the thalamus, relates the terminology of various authors in a most complete way, and discusses the differences in results produced by different experimental techniques. He then goes on to discuss certain of the physiological studies relating the thalamic nuclei to both the cortex and peripheral receptors, with an emphasis on somatotopic localization within the thalamic nuclei. The second portion of this monograph deals with the hypothalamus in much the same way. Here the emphasis is less on the autonomic and endocrine effects of this portion of the brain than on the relationships between the hypothalamus and cortical structures. The relationships between the hypothalamus and the brain stem, spinal cord, and even peripheral nerves are discussed. If the reader is disappointed at the absence of a discussion, or even citation, of certain well known papers in the Western literature, particularly some published in English, he is more than rewarded by finding extensive references to the Eastern European and Russian literature, particularly the work of Bykov and of Sager himself.

R. J. Gumnit

TABLES DE MATIÈRES

Par noms d'auteurs

Tome XCIX – 1961

EPILEPSIA

Journal of the International League against Epilepsy

Fourth Series *Vol. 2 No. 2* *June 1961*

CONTENTS

Annual subscription rate: £ 2.17.0 *or* $ 8.00 *or Dfl.* 30.—

ELSEVIER PUBLISHING COMPANY

AMSTERDAM

ARCHIVES ITALIENNES DE BIOLOGIE

SUMMAIRE DU FASCICULE 4, VOLUME XCIX

Direttore responsabile: Prof. Giuseppe Moruzzi
Editore: F. Vallerini - Pisa
Stabilimenti tipografici «E. Ariani» e «L'Arte della Stampa» - Firenze
Autorizzazione del Tribunale di Pisa N. 20/1956 del 1° Dicembre 1956